The Royal Wolverhampton NHS Trust

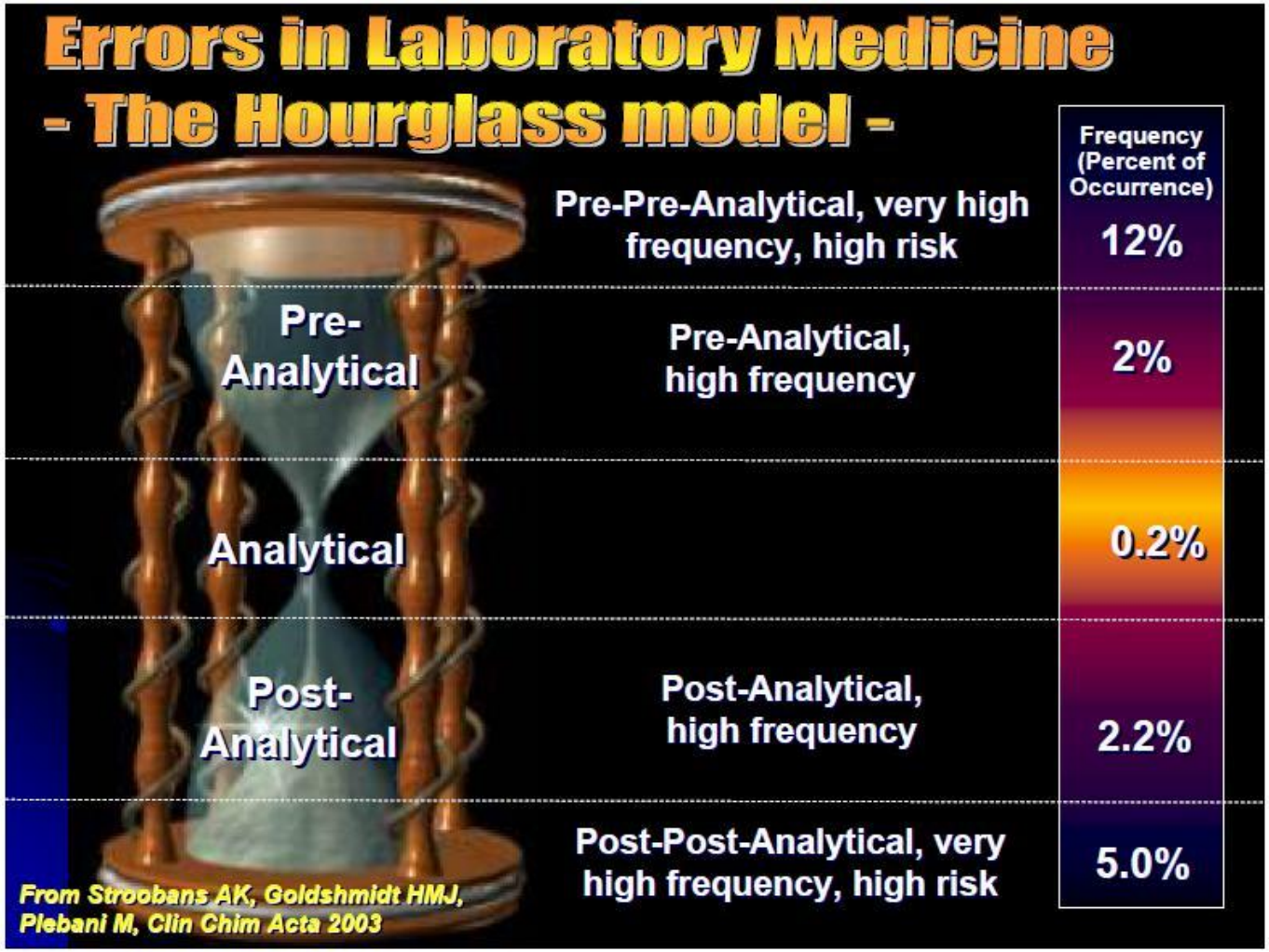
The order of draw - myth or science?

Dr Mike Cornes: Principal Clinical Scientist
Royal Wolverhampton NHS Trust

Where is Wolverhampton?

The Royal Wolverhampton NHS
NHS Trust

Samples Errors



Where is Order of draw Important?

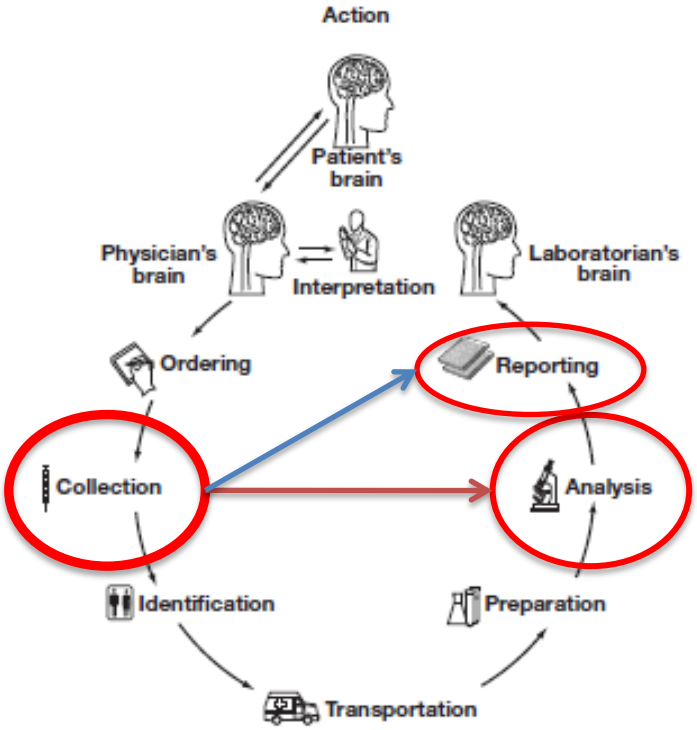


Figure 1 The brain-to-brain loop for laboratory testing 40 years later.

The Brain-to-Brain Loop Concept for Laboratory Testing 40 Years After Its Introduction

Mario Plebani, MD,[1] Michael Laposata, MD, PhD,[2] and George D. Lundberg, MD[3]

Problems Associated with incorrect Order of Draw

- Hypernatremia → Sodium Citrate / NaEDTA
- Hyperkalaemia
- Hypocalcaemia
- Hypomagnesaemia
- Low Zinc
- Low Iron
- Low ALP

kEDTA

- Poor coagulation → transfer of anticoagulants
- Dilution effects → tipping of samples

Case

- Sodium 136 mmol/L (135 - 145)
- Potassium 7.1 mmol/L (3. 5- 5.0)
- Urea 5.1 mmol/L (1.0 -7.0)
- Creatinine 71 μmol/L (60 – 120)
-
- Calcium 1.39 mmol/L (2.10- 2.60)
- Alk Phos 35 IU/ L (30 –130)
- Albumin 40 g/L (36 – 52)

Cause

- Suspected potassium EDTA contamination

Order of Draw

- Originated based on a paper from 1982 by Calam and Cooper
- Still referenced in CLSI guidelines.

Recommended "Order of Draw" for Collecting Blood Specimens into Additive-Containing Tubes

To the Editor:

The problem of interferences in laboratory methods is well documented (*1*), as is the importance of correct procedures for collecting and handling blood specimens (*2–5*).

Here, we direct attention to a problem that can occur when blood is collected into a tube containing an additive just before blood is collected into a tube containing no additives, and we emphasize the need to adhere to the correct "order of draw" when different tubes are used for multiple blood sampling from a single venipuncture.

subject. The first tube, an SST, was immediately followed by an EDTA-K_3 tube. Next we reversed the order of draw for a second venipuncture and a second pair of specimens from the control. As expected, the results for the two EDTA-K_3 tubes had the potassium increased and the calcium suppressed. Reversing the order of draw had no apparent contaminating effect on the results for the SST tube, which suggests that contamination is most likely when there is difficulty with the venipuncture, as was noted in our five cases. Nevertheless, we recommend that specimens should *always* be drawn in nonadditive tubes before additive tubes, to obviate possible contamination.

Clin Chem. 1982 Jun;28(6):1399

CLSI Guidelines

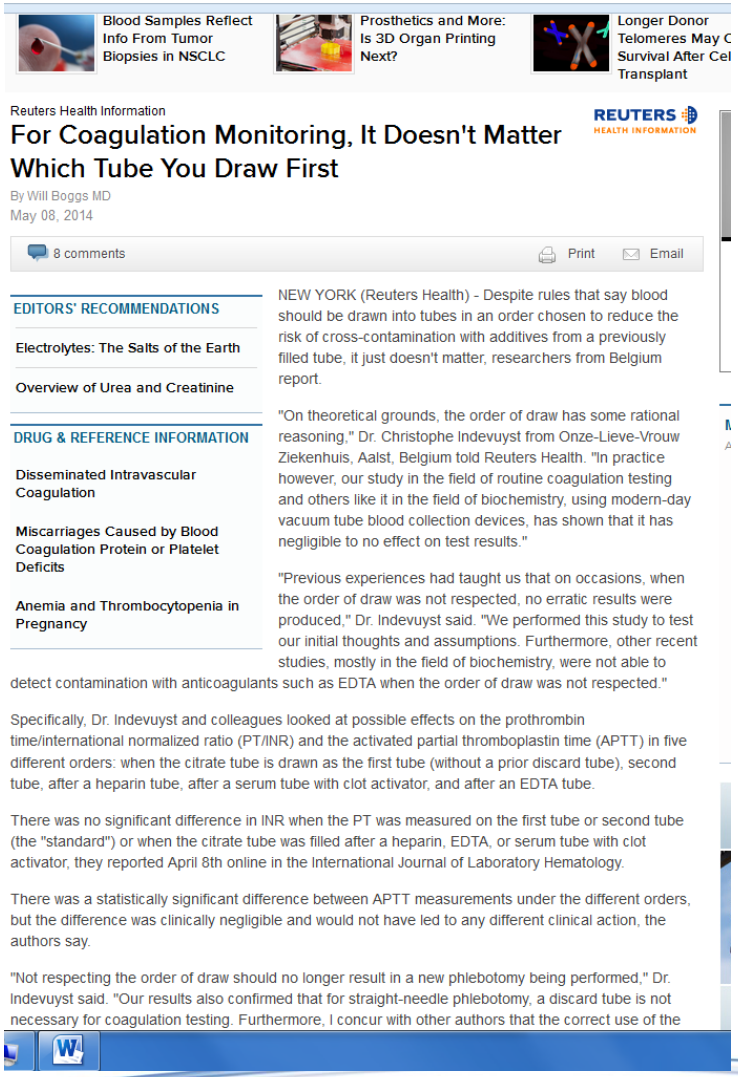
8.10 Step 10: Order of Draw

The following order of draw is recommended for both glass and plastic venous collection tubes when drawing multiple specimens for clinical laboratory testing during a single venipuncture. Its purpose is to avoid possible test result error due to additive carryover.[50,51] All additive tubes should be filled to their stated volumes (see Section 8.9.1[10]).

(1) Blood culture tube
(2) Coagulation tube (eg, blue closure)
(3) Serum tube with or without clot activator, with or without gel (eg, red closure)
(4) Heparin tube with or without gel plasma separator (eg, green closure)
(5) EDTA tube with or without gel separator (eg, lavender closure, pearl closure)
(6) Glycolytic inhibitor (eg, gray closure)

Is Order of Draw a Myth?

Controversy!



Blood Samples Reflect Info From Tumor Biopsies in NSCLC

Prosthetics and More: Is 3D Organ Printing Next?

Longer Donor Telomeres May C Survival After Cel Transplant

Reuters Health Information

REUTERS HEALTH INFORMATION

For Coagulation Monitoring, It Doesn't Matter Which Tube You Draw First

By Will Boggs MD

May 08, 2014

8 comments Print Email

EDITORS' RECOMMENDATIONS

Electrolytes: The Salts of the Earth

Overview of Urea and Creatinine

DRUG & REFERENCE INFORMATION

Disseminated Intravascular Coagulation

Miscarriages Caused by Blood Coagulation Protein or Platelet Deficits

Anemia and Thrombocytopenia in Pregnancy

NEW YORK (Reuters Health) - Despite rules that say blood should be drawn into tubes in an order chosen to reduce the risk of cross-contamination with additives from a previously filled tube, it just doesn't matter, researchers from Belgium report.

"On theoretical grounds, the order of draw has some rational reasoning," Dr. Christophe Indevuyst from Onze-Lieve-Vrouw Ziekenhuis, Aalst, Belgium told Reuters Health. "In practice however, our study in the field of routine coagulation testing and others like it in the field of biochemistry, using modern-day vacuum tube blood collection devices, has shown that it has negligible to no effect on test results."

"Previous experiences had taught us that on occasions, when the order of draw was not respected, no erratic results were produced," Dr. Indevuyst said. "We performed this study to test our initial thoughts and assumptions. Furthermore, other recent studies, mostly in the field of biochemistry, were not able to detect contamination with anticoagulants such as EDTA when the order of draw was not respected."

Specifically, Dr. Indevuyst and colleagues looked at possible effects on the prothrombin time/international normalized ratio (PT/INR) and the activated partial thromboplastin time (APTT) in five different orders: when the citrate tube is drawn as the first tube (without a prior discard tube), second tube, after a heparin tube, after a serum tube with clot activator, and after an EDTA tube.

There was no significant difference in INR when the PT was measured on the first tube or second tube (the "standard") or when the citrate tube was filled after a heparin, EDTA, or serum tube with clot activator, they reported April 8th online in the International Journal of Laboratory Hematology.

There was a statistically significant difference between APTT measurements under the different orders, but the difference was clinically negligible and would not have led to any different clinical action, the authors say.

"Not respecting the order of draw should no longer result in a new phlebotomy being performed," Dr. Indevuyst said. "Our results also confirmed that for straight-needle phlebotomy, a discard tube is not necessary for coagulation testing. Furthermore, I concur with other authors that the correct use of the

- Dr Cornes notes that the evidence in support of a strict order of draw is virtually non-existent, but still recommends that this process occurs.
- Just another one of those outdated practices and/or myths in health practice that are so hard to shift?
- draw a CBC before a chemistry AND expect hypocalcemia and hyperkalemia in the chemistry specimen!
- In a large system core laboratory, we see the typical pattern of hyperkalemia/hypocalcemia at least once a week
- We can expect an uptick in those typical hyperkalemia/hypocalcemia cases now as the uninformed nurses spread word that "it doesn't matter".
- The order of draw is an established best practice, and must remain so, if only to not confuse phlebotomists at large.
- as Dr. Cornes says, when ideal phlebotomy is not possible, and since following the "best practice" of established order of draw has no detrimental impact on results, we must continue this as the best practice, especially as it's taken such a long time and practice to get embedded internationally.

Effect of Carryover of Clot Activators on Coagulation Tests During Phlebotomy

Yoko Fukugawa, MA,[1] Hiroaki Ohnishi, MD,[1] Takahiro Ishii,[1] Ayako Tanouchi,[1] Junko Sano,[1] Haruko Miyawaki,[1] Tomonori Kishino, MD,[1] Kouki Ohtsuka, MD,[1] Hideaki Yoshino, MD,[2] and Takashi Watanabe, MD[1]

Results of Coagulation Tests of the First and Second Tubes (Mean ± Standard Deviation)*

	Healthy Volunteers (n = 75)		Patients Taking Warfarin (n = 25)	
	First Tube	Second Tube	First Tube	Second Tube
PT (%)	99.3 ± 3.4	99.4 ± 3.0	45.5 ± 13.5	45.8 ± 12.7
PT ratio	0.96 ± 0.05	0.95 ± 0.05†	1.59 ± 0.25	1.57 ± 0.24‡
PT-INR	0.95 ± 0.06	0.94 ± 0.06†	1.86 ± 0.40	1.84 ± 0.37‡
aPTT (s)	36.9 ± 4.3	36.9 ± 4.2	45.5 ± 7.8	45.2 ± 7.6
Fibrinogen (mg/dL)	311.9 ± 68.3	314.0 ± 69.6	352.3 ± 68.4	362.4 ± 75.5†
DD (μg/mL)	0.28 ± 0.18	0.28 ± 0.18	0.90 ± 1.77	0.93 ± 1.76
FMC (μg/mL)	2.90 ± 1.66	3.42 ± 3.35	4.05 ± 6.09	4.09 ± 5.84

aPTT, activated partial thromboplastin time; DD, D-dimer; FMC, fibrin monomer complex; PT, prothrombin time; PT-INR, prothrombin time–international normalized ratio;
* The first and second tubes were drawn before and after the serum tube, respectively.
† $P < .01$.
‡ $P < .05$.

In conclusion, the present study suggests that the carryover effect of the clot activators in the serum tubes on major coagulation tests is minimal in the clinical setting. Therefore, a "coagulation after serum" blood draw sequence may be acceptable when standard phlebotomy procedures are used. Further studies including a large number of patients and the use of other coagulation tests are needed to verify the feasibility of a "coagulation after serum" blood draw sequence.

Am J Clin Pathol. 2012 Jun;137(6):900-3

The order of draw: much ado about nothing?

C. INDEVUYST, W. SCHUERMANS, E. BAILLEUL, P. MEEUS

Table 2. Summary of the results

	PT (INR)		APTT (s)	
	Median (IQR)	Mean bias (95% CI) 95% significance	Median (IQR)	Mean bias (95% CI) 95% significance
Phase 1				
Reference	2.6 (2.1–3.0) (n = 95)		33.9 (31.6–37.0) (n = 95)	
First tube	2.6 (2.1–3.0) (n = 95)	0.001579 (−0.02008 to 0.02323) P = 0.6205	34.5 (31.8–37.0) (n = 95)	0.1379 (−0.04729 to 0.3231) P = 0.0227
After heparin	2.6 (2.1–3.0) (n = 94)	0.002021 (−0.01107 to 0.01511) P = 0.4915	33.7 (31.4–36.7) (n = 93)	−0.1742 (−0.4069 to 0.05855) P = 0.2668
Phase 2				
Reference	2.7 (2.2–3.3) (n = 91)		34.0 (31.4–37.0) (n = 93)	
After EDTA	2.7 (2.2–3.3) (n = 91)	−0.01264 (−0.04161 to 0.01633) P = 0.7603	34.0 (31.2–36.8) (n = 93)	−0.2249 (−0.4012 to −0.04870) P = 0.0016
After serum	2.7 (2.2–3.3) (n = 91)	0.007033 (−0.01554 to 0.02961) P = 0.3388	34.4 (32.0–37.4) (n = 93)	0.2180 (0.032860 to 0.4032) P = 0.0215

PT, prothrombin time; INR, international normalized ratio (reference range: <1.2); APTT, activated partial thromboplastin time (reference range: 24–31 s); CI, confidence interval; IQR, interquartile range.
P-values derived from Wilcoxon signed rank test.

Int J Lab Hematol. 2015 Feb;37(1):50-5

The order of draw of blood specimens into additive containing tubes does not affect potassium and calcium measurements

A Majid, D C Heaney, N Padmanabhan, R Spooner

Table 1 Mean concentration differences between first and final blood samples for control and trial subjects. Results are expressed as mean difference (SD)

	n	*Potassium (mmol/l)*	*Calcium (mmol/l)*
Control	12	0.025 (0.205)	0.014 (0.041)
Trial	34	0.015 (0.131)	−0.011 (0.044)

Table 2 T tests between paired and unpaired analyte concentrations

	Potassium	*Calcium*
Control pre *v* post	p = 0.68	p = 0.26
Trial pre *v* post	p = 0.52	p = 0.15
Control *v* trial	p = 0.87	p = 0.09

from damaged cells and this high extracellular potassium leads to depolarisation of local cells causing calcium to flood into them, resulting in high potassium and low calcium measurements.

We conclude that the order of draw does not effect the potassium and calcium measurements but difficult venepuncture may result in high potassium and low calcium concentrations as a result of local factors.

J Clin Pathol. 1996 Dec;49(12):1019-20

Gianluca Salvagno, Gabriel Lima-Oliveira, Giorgio Brocco, Elisa Danese, Gian Cesare Guidi and Giuseppe Lippi*

The order of draw: myth or science?

Table 1 Results (median and IQR) and bias (mean and 95% confidence interval) of potassium, sodium, calcium, magnesium, and phosphorus measured in serum tubes collected before or after either a K_2-EDTA or sodium citrate tube.

	Before	After	Bias
K_2-EDTA tube			
Potassium, mmol/L	4.40 (4.17 to 4.62)	4.45 (4.24 to 4.68), p=0.064	0.04 (-0.01 to 0.08), p=0.127
Sodium, mmol/L	143 (142 to 144)	143 (142 to 144), p=0.091	0.2 (-0.1 to 0.4), p=0.182
Calcium, mmol/L	2.41 (2.35 to 2.46)	2.41 (2.36 to 2.46), p=0.095	0.00 (0.00 to 0.01), p=0.190
Magnesium, mmol/L	0.85 (0.81 to 0.89)	0.84 (0.81 to 0.87), p=0.127	-0.01 (-0.02 to 0.01), p=0.253
Phosphorus, mmol/L	1.06 (0.97 to 1.16)	1.06 (0.97 to 1.16), p=0.070	0.00 (0.00 to 0.01), p=0.141
Sodium citrate tube			
Potassium, mmol/L	4.50 (4.27 to 4.87)	4.54 (4.34 to 4.95), p=0.058	0.04 (0.00 to 0.08), p=0.056
Sodium, mmol/L	144 (142 to 145)	144 (142 to 145), p=0.170	0.1 (-0.1 to 0.4), p=0.341
Calcium, mmol/L	2.38 (2.32 to 2.44)	2.38 (2.33 to 2.45), p=0.054	0.01 (0.00 to 0.02), p=0.108
Magnesium, mmol/L	0.85 (0.80 to 0.88)	0.84 (0.80 to 0.88), p=0.231	0.00 (-0.01 to 0.01), p=0.462
Phosphorus, mmol/L	1.05 (0.94 to 1.18)	1.04 (0.94 to 1.20), p=0.063	0.00 (0.00 to 0.01), p=0.126

Clin Chem Lab Med 2013 Dec;51(12):2281-5

Our Studies! – Adding EDTA analysis.

Short report

Effect of order of draw of blood samples during phlebotomy on routine biochemistry results

Raashda A Sulaiman,[1] Michael P Cornes,[1] Simon J Whitehead,[1] Nadia Othonos,[1] Clare Ford,[1] Rousseau Gama[1,2]

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

Analyte	Posture	Sample taken before EDTA	Sample taken after EDTA	p Value
EDTA (mmol/l)	Sitting	<0.2	<0.2	1.0
	Lying	<0.2	<0.2	1.0
Potassium (mmol/l)	Sitting	4.1 (0.25)	4.2 (0.30)	0.5126
	Lying	4.1 (0.30)	4.1 (0.25)	0.7690
Adjusted calcium (mmol/l)	Sitting	2.20 (0.06)	2.23 (0.07)	0.2633
	Lying	2.18 (0.05)	2.20 (0.07)	0.3916
Magnesium (mmol/l)	Sitting	0.79 (0.05)	0.81 (0.05)	0.3540
	Lying	0.80 (0.07)	0.80 (0.05)	0.8272
Zinc (μmol/l)	Sitting	13.32 (2.58)	13.94 (2.56)	0.5586
	Lying	13.00 (2.52)	13.17 (2.55)	0.8736
Alkaline phosphatase (IU/l)	Sitting	64.31 (18.59)	66.08 (19.64)	0.8247
	Lying	64.00 (17.87)	64.42 (18.37)	0.9556
Iron (mmol/l)	Sitting	15.93 (3.91)	16.12 (4.02)	0.9030
	Lying	15.54 (4.09)	15.67 (4.17)	0.9378

Results are expressed as mean (SD).

J Clin Pathol. 2011 Nov;64(11):1019-20

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

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

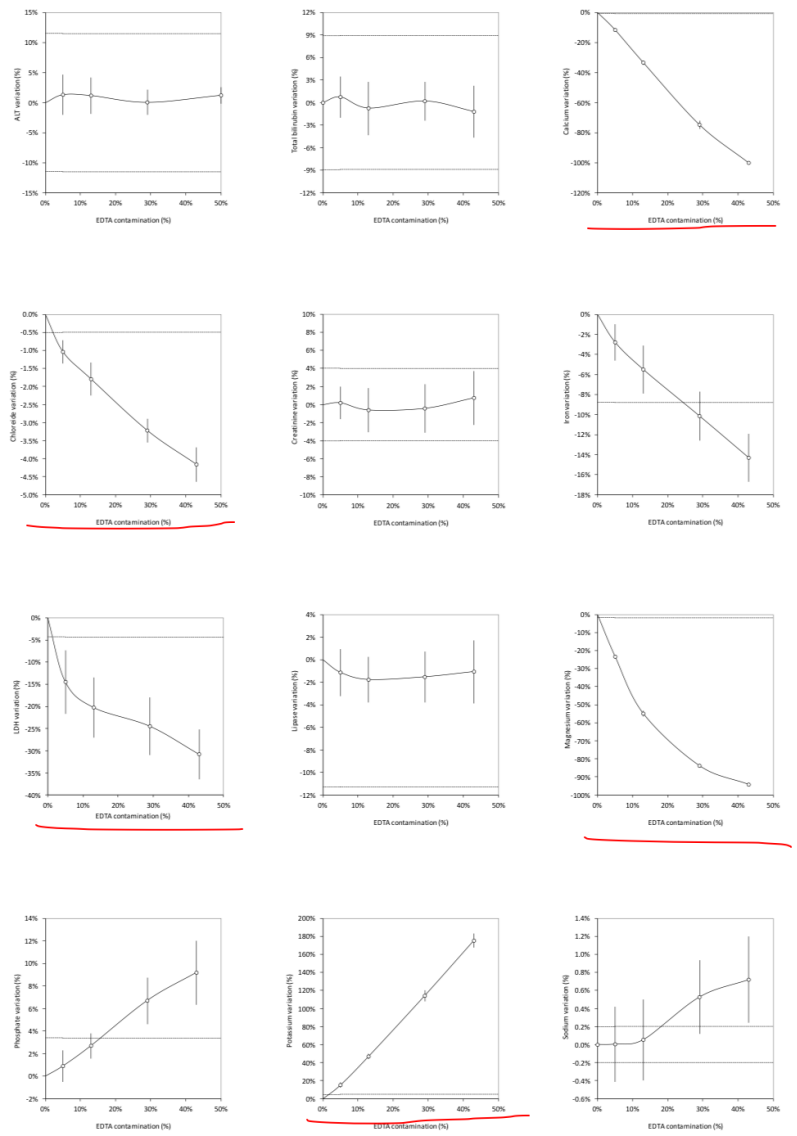
Results expressed as mean (SD)

CONCLUSIONS

- Incorrect order of draw under ideal phlebotomy conditions does not cause EDTA contamination irrespective of closed blood collection systems

Br J Biomed Sci. 2012;69(3):136-8

The Science! (Or Reality)



The Royal Wolverhampton NHS Trust

Contamination of lithium heparin blood by K2-ethylenediaminetetraacetic acid (EDTA): an experimental evaluation

Gabriel Lima-Oliveira*[1,2], Gian Luca Salvagno[1], Elisa Danese[1], Giorgio Brocco[1], Gian Cesare Guidi[1,2], Giuseppe Lippi[3]

[1]Laboratory of Clinical Biochemistry, Department of Life and Reproduction Sciences, University of Verona, Verona, Italy
[2]Post-Graduate Program of Pharmaceutical Sciences, Department of Clinical Analyses, Federal University of Parana, Curitiba, Parana, Brazil
[3]Laboratory of Clinical Chemistry and Hematology, Academic Hospital of Parma, Parma, Italy

- Contamination by even small amounts (5%) of EDTA affects Calcium, Magnesium, potassium Chloride and LDH
- Iron, phosphate and Sodium are ok up to 30%

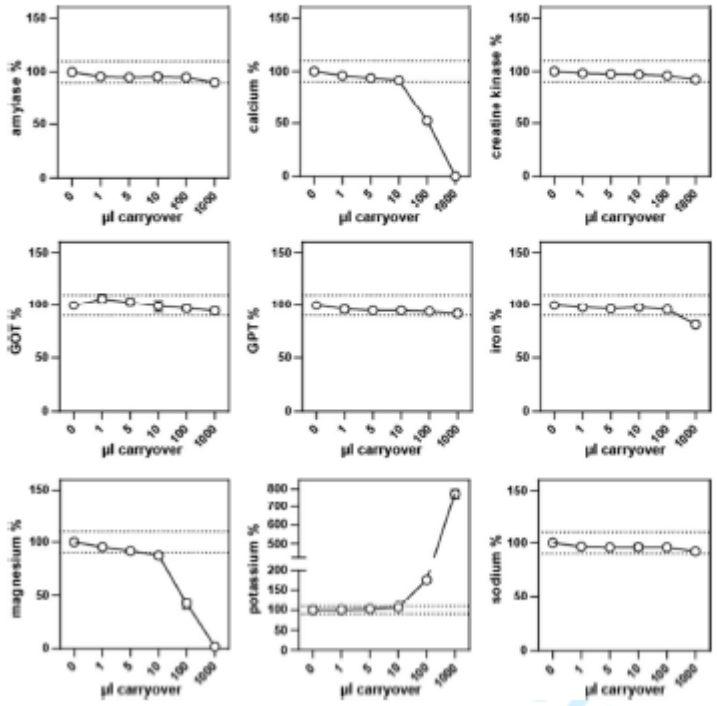
Biochem Med (Zagreb). 2014 Oct 15;24(3):359-67

Relevance of EDTA carryover during blood collection.

Clin Chem Lab Med. 2015 Jan 23. pii: /j/cclm.ahead-of-print/cclm-2014-0944/cclm-2014-0944.xml. doi: 10.1515/cclm-2014-0944. [Epub ahead of print]

Cadamuro J, Felder TK, Oberkofler H, Mrazek C, Wiedemann H, Haschke-Becher E.

Figure1 - Changes of biomarker values after a simulated carryover of 1µl, 5µl, 10µl, 100µl and 1000µl of K_3EDTA whole-blood.



Sodium citrate contamination.

Table 1 Influence of contaminants on electrolyte results

	Patient		Control 1			Control 2			Control 3		
	Initial	Repeat	Baseline	Coagulation	ESR	Baseline	Coagulation	ESR	Baseline	Coagulation	ESR
Sodium (mmol/L)	170	137	141	167	182	141	165	174	143	168	180
AVL:sodium (mmol/L)	145	135	139	152	152	138	150	148	142	151	149
Potassium (mmol/L)	2.7	4.0	4.4	3.6	3.0	4.2	4.0	3.7	3.8	2.8	2.6
AVL:potassium (mmol/L)	2.5	4.0	4.6	3.3	2.6	4.3	3.7	3.2	3.8	2.8	2.3
Urea (mmol/L)	5.5	8.3	5.2	5.0	4.1	5.1	4.6	4.2	6.6	5.6	5.3
Creatinine (μmol/L)	46	52	86	55	55	4.6	38	32	101	82	72
Chloride (mmol/L)	64	103	99	81	54	104	87	66	107	89	66
Glucose (mmol/L)	4.8	5.3	5.5	–	4.1	4.9	4.5	4.1	4.7	4.1	3.8
Osmolality (mmol/L)	275	291	294	296	271	295	300	284	297	297	280
Calculated osmolality (mmol/L)	**350**	**295**	292	343*	372	292	339	356	297	346	369
Osmolar gap (mmol/L)	**−75**	**−4**	+2	−47	−99	+3	−39	−72	0	−49	−89

Undetected spurious hypernatraemia wastes health-care resources

Michael P Comes[1], Clare Ford[1] and Rousseau Gama[1,2]

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Corresponding author: Dr Michael Comes. Email: Michael.cornes@nhs.net

Ann Clin Biochem. 2011 Jan;48(Pt1):87-8

Kahena Bouzid*, Ahlem Bartkiz, Aymen Bouzainne, Samia Cherif, Saddem Ramdhani, Aida Zairi, Mehdi Mrad, Afef Bahlous and Jaouida Abdelmoula

How to reduce EDTA contamination in laboratory specimens: a Tunisian experience

Table 1 Frequency of EDTA contamination identified by the laboratory before and after the awareness campaign.

Before awareness campaign, 1 January to 31 March 2014		After awareness campaign, 8 April to 7 May 2014	
Total cases of hyperkalemia	EDTA contamination, n (percentage)	Total cases of hyperkalemia	EDTA contamination, n (percentage)
297	132 (44.4)	48	13 (27.0)*

*Significantly different from the percentage before awareness campaign, with $p<0.05$.

EDTA contamination was defined as the presence of hyperkalemia (when serum potassium was over 5.8 mmol/L), hypocalcemia (when serum adjusted calcium was < 2.00 mmol/L), and hypomagnesemia (when serum magnesium was < 0.66 mmol/L) with normal renal function.

Clin Chem Lab Med 2015 Jan;53(1):e9-e12

Case report

Incorrect order of draw could be mitigate the patient safety: a phlebotomy management case report

Gabriel Lima-Oliveira*[1,2,3,4], Giuseppe Lippi[5], Gian Luca Salvagno[1], Martina Montagnana[1], Geraldo Picheth[2], Gian Cesare Guidi[1,2]

Table 1. Laboratory results.

Parameter	Reference interval	Critical values (lower limit/ upper limit)	1st blood collection		2nd blood collection		Mean % difference		RCV	CVa	CVw
			Serum vacuum tube	Dedicated syringe	Serum vacuum tube	Dedicated syringe	Serum vacuum tube	Dedicated syringe			
Total cholesterol (mmol/L)	4.22-7.12	NA	4.48	-	4.50	-	-0.44	-	15.69	1.7	5.4
HDL-cholesterol (mmol/L)	0.78-1.66	NA	1.04	-	1.03	-	0.96	-	22.73	4.1	7.1
Triglycerides (mmol/L)	0.64-3.16	NA	3.63	-	3.60	-	0.83	-	58.17	1.9	20.9
Sodium (mmol/L)	136-145	120/160	138	138	139	139	-0.72	-0.72	3.16	0.9	0.7
Potassium (mmol/L)	3.5-5.1	2.8/6.2	4.8	**8.5**	4.7	4.5	2.08	**47.06**	13.86	1.4	4.8
Total calcium (mmol/L)	2.15-2.55	1.50/3.25	2.36	1.48	2.37	2.38	-0.42	**-60.81**	5.42	0.6	1.9
Free calcium (mmol/L)	1.15-1.33	0.75/1.60	-	**0.15**	-	1.21	-	**-706.67**	5.00	0.6	1.7
Urea (mmol/L)	2.1-7.1	NA/28.6	5.3	-	5.2	-	1.89	-	34.79	2.5	12.3
Creatinine (µmol/L)	80-115	NA/442	84	-	85	-	-1.19	-	17.91	2.4	6.0
Total protein (g/L)	64-83	NA	78	-	77	-	1.28	-	8.08	1.1	2.7
Albumin (g/L)	35-52	NA	45	-	46	-	-2.22	-	9.12	1.1	3.1
Alkaline phosphatase (µkat/L)	0.71-1.67	NA	1.28	-	1.26	-	1.56	-	19.04	2.5	6.4

Bold values are results outside of critical limits (11,12) and bold mean % differences represent clinically significant variations, when compared with reference change value (RCV).
NA - not applied; CVa - analytical within run precision of the internal quality control, CVw - coefficient of variation within-subject (13).

Biochem Med (Zagreb). 2013;23(2):218-23

Spurious hyperkalaemia due to EDTA contamination: common and not always easy to identify

Michael P Cornes[1], Clare Ford[1] and Rousseau Gama[1,2]

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Corresponding author: Mr Michael Cornes. Email: cornesmp@aol.com

Results: Twenty-eight out of 117 hyperkalaemic samples were contaminated with EDTA. Only serum zinc values below the reference range had 100% sensitivity for indicating EDTA contamination, but even at an optimal specificity of 89% at least 12 potentially genuine hyperkalaemic samples would be rejected.

EDTA sample contamination is common and often undetected, putting patients at unnecessary risk of harm

C. L. Sharratt,[1] C. J. Gilbert,[1] M. C. Cornes,[1] C. Ford,[1] R. Gama[1,2]

Table 1 EDTA +ve samples detected by an audit of hypocalcaemic, hypomagnesaemic and hypozincaemic samples and by routine screening of unexplained hyperkalaemic samples (serum potassium > 6.0 mmol/l)

EDTA + samples	Calcium < 2.00 mmol/l	Zinc < 11.0 μmol/l	Magnesium < 0.7 mmol/l	Potassium > 5.0 mmol/l*	EDTA, mmol/l
Audit (n = 22)	19 (86.3%)	21 (95.4%)	15 (68.1%)	13 (61.9%) (n = 21)	0.32 (0.23–0.41)
Routine screening (n = 9)	9 (100%)	9 (100%)	9 (100%)	9 (100%)	0.50 (0.50–0.60)
Total (n = 31)	28 (90.3%)	30 (96.7%)	24 (77.4%)	22 (71.0%) (n = 30)	0.41 (0.27–0.50)

*K not measured in one sample because of delayed sample separation. EDTA results are medians (interquartile range). EDTA, ethylenediaminetetraacetic acid.

Ann Clin Biochem. 2008 Nov;45(Pt 6):601-3 / Int J Clin Pract. 2009 Aug;63(8): 1259-62

Multi-Centre Observational Study of Spurious Hyperkalaemia Due to EDTA Contamination

MICHAEL P CORNES [1], FRASER DAVIDSON [3], LUCY DARWIN [4], CHRIS GAY [5]
MARK REDPATH [6], JENNA L WALDRON [7], CLARE FORD [1], ROUSSEAU GAMA [1,2]

- All hyperkalaemic samples over a 1 month period from 5 different hospitals covering 3 different tube manufacturers were analysed

Table 1. Prevalence of spurious hyperkalaemia due EDTA contamination in one month in five different laboratories.

Lab	Tube Type	U&Es	Hyperkalaemic	EDTA +ve	EDTA +ve Identified by lab staff	%Hyperkalaemic EDTA +ve
1	Sarstedt	26201	300	20	NA	6.7
2	Sarstedt	23818	110	5	NA	4.6
3	Greiner	32764	200	3	0	1.5
4	Greiner	18697	163	2	1	1.2
5	B-D	30344	140	7	4	5.0
Total	-	131824	913	37	-	4.1

BD = Becton-Dickinson
NA = Not applicable because of routine measurement of EDTA in hyperkalaemic samples

- Gross contamination is easy to identify
- Modest EDTA causing spurious hyperkalaemia can only confidently be identified by measuring EDTA

Clin Lab. 2010;56(11-12):597-9

What are the mechanisms of EDTA contamination.

- 3 possible mechanisms
  1) Direct transfer
     - Easily identified
  2) Backflow due to incorrect order of draw
     - Appears not to be the case under ideal phlebotomy conditions
  3) Syringe needle contamination
     - Best current hypothesis when combined with incorrect order of draw

What is the source of contamination? - Hypothesis

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23 September 2011 Last updated at 01:06

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Call for more training to improve blood tests in A&E

By Adam Brimelow
Health Correspondent, BBC News

Scientists say doctors need better training to avoid mistakes in blood samples taken in hospital A&E departments.

The warning from the Association for Clinical Biochemistry follows an audit at Birmingham City Hospital.

The trust has put in place extra training, but the ACB says this is a problem across the UK.

Can doctors take a blood sample?

The College of Emergency Medicine says it is essential that staff use the right technique to collect blood.

Blood test results are often key to assessing patients when they arrive in

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What is the source of contamination? - Hypothesis

- Hypothesis: Is it is due to syringe transfer?

Table 1 Variation in phlebotomy technique practised in the Majors area of the Emergency Medicine Department

Technique	Number
Cannula with syringe	19 (38%)
Cannula with evacuated tubes and adaptor	21 (42%)
Syringe and needle into vein	7 (14%)
Evacuated tubes system conventionally used	3 (6%)

52% of samples taken with a syringe

Table 2 How blood tubes are filled when they are not the primary receiver of samples

Method of tube filling	Number
Cannula with syringe	
Needle added and then tube cap pierced	14 (74%)
Evacuated tube cap removed	5 (26%)
Syringe and needle into vein	
Needle kept on and tube caps pierced	6 (86%)
Needle removed and evacuated tube cap removed	1 (14%)
Both methods	
Needle piercing of tube cap	20 (77%)
Needle and tube cap removed	6 (23%)

All of these can potentially lead to contamination if an incorrect order of draw is performed.

Ann Clin Biochem. 2011 Nov;48(Pt6):562-5

What is the source of contamination? - Hypothesis

- Hypothesis: Is it is due to syringe transfer?

Table 4 Order of fill of evacuated tubes with particular reference to the potassium-EDTA and biochemistry tube ($n = 49$)

Tube type	Filled first
Serum tube – biochemistry	27 (55%)
Potassium-EDTA – haematology	20 (41%)
Serum tube first but more blood added after EDTA tube filled	2 (4%)

Ann Clin Biochem. 2011 Nov;48(Pt6):562-5

What Should we do?

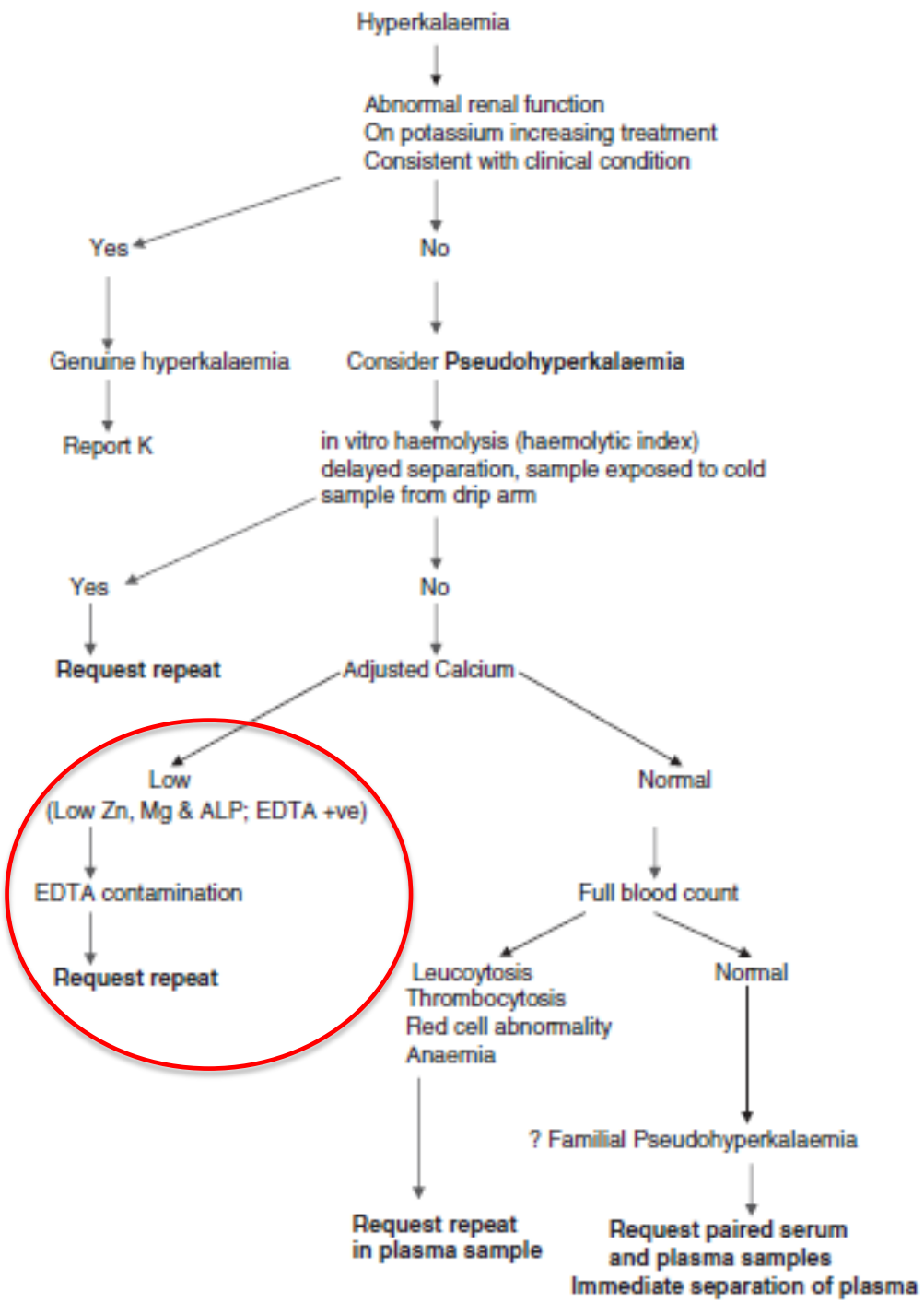
- Develop Assays
  - EDTA assay was developed in house to aid the detection of contamination
- Develop Algorithms
  - Surrogate markers for EDTA if no assay

Table 1 Usefulness of surrogate markers at the lower limit of their reference ranges for detecting ethylenediaminetetra acetic acid contamination

Analyte	Cut-off value	Sensitivity (%)	Specificity (%)	Reference range
Calcium	2.10 mmol/L	86	92	2.10–2.65 mmol/L
Zinc	11 μmol/L	100	63	11–25 μmol/L
Magnesium	0.70 mmol/L	80	95	0.70–1.20 mmol/L
Alkaline phosphatase	38 IU/L	29	99	38–126 IU/L

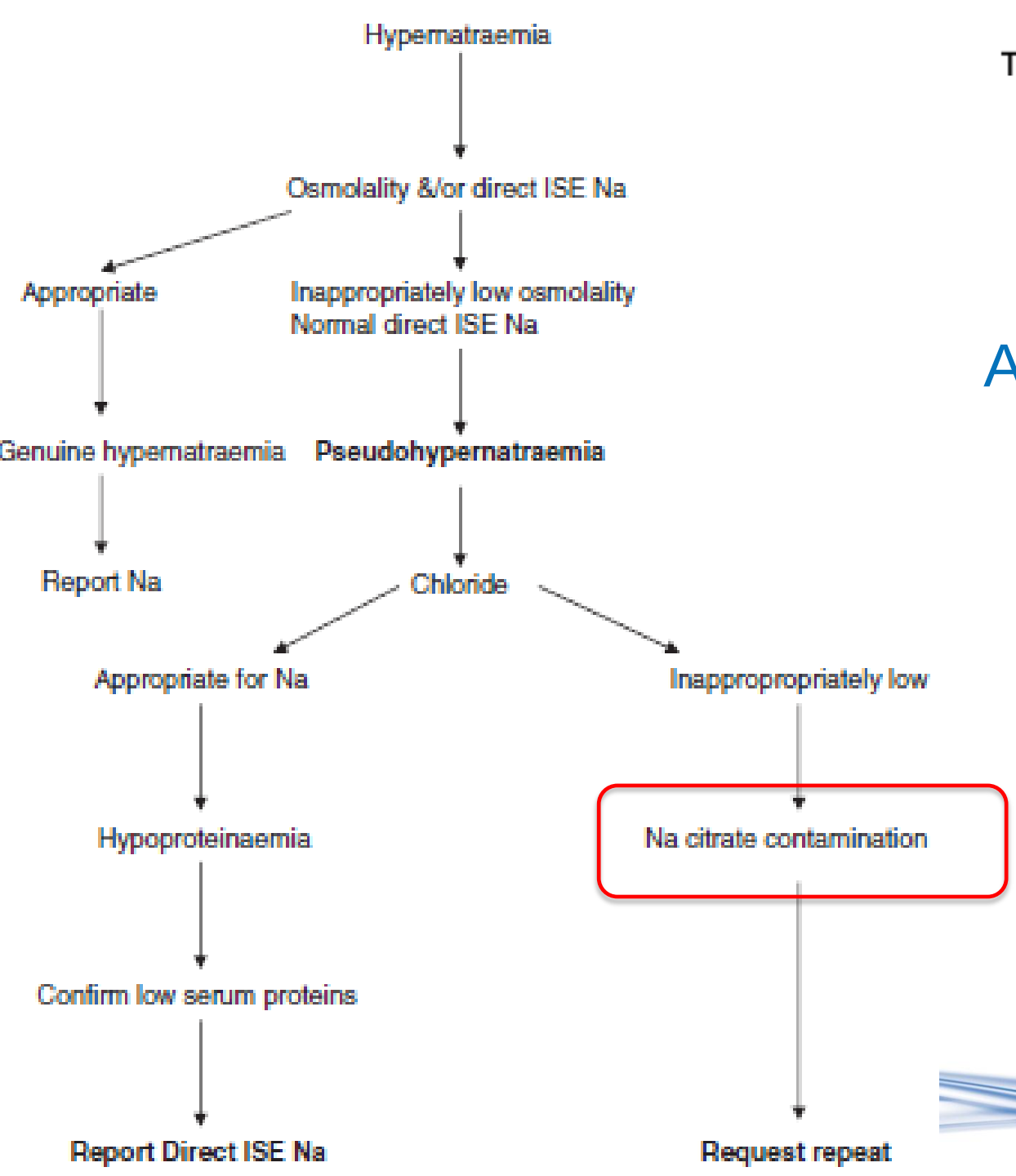
Ann Clin Biochem. 2008 Nov;45(Pt 6):601-3

Algorithms – High Potassium



Clin Chim Acta. 2011 Jan 14;412(1-2):1-6

Algorithms – High Sodium



Conclusions

- Many opinions on Order of Draw.
- Reverse order of draw using closed loop venesection systems as a cause of sample cross-contamination is a myth.
- Science shows that it still occurs
- There is no disadvantage in following a set order of draw.
- Sample cross-contamination is not uncommon and further studies are required to investigate and confirm other mechanisms of sample cross-contamination in order to implement focused appropriate preventive measures.

Thanks for listening.

- Questions?
  - Michael.cornes@nhs.net

Question 1

- How do you currently spot EDTA contamination?
  - Specific assay?
  - Surrogate markers?
  - Chance (unbelievable results.
  - We don't do anything

Question 2 – What could explain this?

- **GP sample**
  - Indirect Na 170mmol/L
  - Direct Na 145mmol/L
  - K 2.7mmol/L
  - Urea 5.5mmol/L
  - Creatinine 46umol/L
  - Glucose 4.8 mmol/L
  - Cl 64mmol/L
  - Osmo 272mOsm/Kg (Calculated = 355)
- **Repeat of bloods in 2° care**
  - Indirect Na 137mmol/L
  - Direct Na 135mmol/L
  - K 4.0mmol/L
  - Urea 8.3mmol/L
  - Creatinine 52umol/L
  - Cl 103mmol/L
  - Osmo 290mOsm/Kg (Calculated = 295.6)

Answers

- A – Hypoproteinamia
- B - Sample contamination
- C – Sample Mix up
- D – Faulty Equipment
- E – Don’t Know

Question 3

- Is Order of Draw still important in modern practice?
  - A = Yes
  - B = No
  - C = Still Unsure