

## Opinion Paper

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# Order of blood draw: Opinion Paper by the European Federation for Clinical Chemistry and Laboratory Medicine (EFLM) Working Group for the Preanalytical Phase (WG-PRE)

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**Abstract:** It has been well reported over recent years that most errors within the total testing process occur in the pre-analytical phase (46%–68.2%), an area that is usually outside of the direct control of the laboratory and which includes sample collection (phlebotomy). National and international (WHO, CLSI) guidelines recommend that the order of draw of blood during phlebotomy should be blood culture/sterile tubes, then plain tubes/gel tubes, then tubes containing additives. This prevents contamination of sample tubes with additives from previous tubes that could cause erroneous results. There have been a number of studies recently looking at whether order of draw remains a problem with modern phlebotomy techniques and materials, or it is an outdated practice followed

simply because of historical reasons. In the following article, the European Federation of Clinical Chemistry and Laboratory Medicine Working Group for the Preanalytical Phase (EFLM WG-PRE) provides an overview and summary of the literature with regards to order of draw in venous blood collection. Given the evidence presented in this article, the EFLM WG-PRE herein concludes that a significant frequency of sample contamination does occur if order of draw is not followed during blood collection and when performing venipuncture under less than ideal circumstances, thus putting patient safety at risk. Moreover, given that order of draw is not difficult to follow and knowing that ideal phlebotomy conditions and protocols are not always followed or possible, EFLM WG-PRE supports the continued recommendation of ensuring a correct order of draw for venous blood collection.

**Keywords:** order of draw; patient safety; phlebotomy; pre-analytical phase; preanalytical quality.

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## Introduction

The total testing process in laboratory medicine encompasses every step in a cycle from the clinician's decision to request the test, through venous blood collection all the way to receipt of the result by the initial requestor, i.e. the brain-to-brain loop [1]. It has been well reported over recent years that only 7%–13% of errors in the total testing process occur in the analytical phase. Most errors occur in the pre-analytical phase (46%–68.2%), a phase that is usually outside of the control of the laboratory and includes sample collection (phlebotomy) [2–4]. It is important that every step in the total testing process is

performed using the correct and standardised procedure to ensure the accuracy of results produced and to ensure the best patient care pathway is achieved.

National and international (WHO, CLSI) guidelines recommended that the order of draw of blood during phlebotomy should be blood culture/sterile tubes, then coagulation tubes, then plain tubes/gel tubes, then tubes containing additives (Table 1) [5, 6]. This prevents contamination of sample tubes with additives from previous tubes that could cause erroneous results, for example sodium citrate or more commonly potassium ethylenediaminetetraacetic acid (kEDTA).

These recommendations are based on a case report and follow-up study of only five subjects by Calam and Cooper [7], who reported that incorrect order of draw using non-evacuated blood collection systems caused hyperkalaemia and hypocalcaemia, which are surrogate markers of *in vitro* kEDTA sample contamination. The authors did, however, acknowledge that contamination with additives only occurred during difficult venipuncture and could not be replicated under ideal phlebotomy conditions [7]. As well as hyperkalaemia and hypocalcaemia there are various other potential consequences of incorrect order of draw. These include:

- Hypernatraemia due to sodium citrate or sodium EDTA contamination
- Hyperkalaemia due to potassium EDTA contamination
- Hypocalcaemia due to EDTA contamination
- Hypomagnesaemia due to EDTA contamination
- Hypozincaemia due to EDTA contamination
- Low iron due to EDTA contamination
- Low alkaline phosphatase (ALP) due to EDTA contamination
- Poor coagulation due to transfer of anticoagulants
- Clot activator transfer interfering with coagulation tests
- Dilution effects due to pouring one sample into another of samples

Most of these are due to direct addition of a contaminant or due to dilution. EDTA acts through binding to divalent

cations, preventing their analysis. ALP may be low due to the binding of EDTA to magnesium, a cofactor for ALP. EDTA binds to these cations with varying affinities with the highest affinity for zinc and the lowest for magnesium, and this can make EDTA contamination potentially difficult to definitively identify especially at low levels using surrogate markers alone [8, 9].

There have been a number of studies recently looking at whether the order of draw remains a problem with modern phlebotomy techniques and materials, or whether it is an outdated practice followed simply because we always have. In this article, the European Federation of Clinical Chemistry and Laboratory Medicine Working Group for the Preanalytical Phase (EFLM WG-PRE) will provide a summary of the evidence and a clear recommendation on the best practice with regards to the order of draw in venous blood collection.

## The evidence

Studies by Fukugawa et al. [10] and Indevuyst et al. [11] both looked at the effect of anticoagulant carryover on various markers of coagulation when phlebotomy was performed using a closed-loop system following the manufacturer's instructions, i.e. the samples are collected directly from the vein into the sample collection tube, not via a syringe. In both studies, they clearly showed that there was no statistically significant difference in results obtained from tubes taken before or after tubes containing anticoagulants. There have also been a number of studies looking at biochemical parameters and order of draw. In 1996, Majid et al. [12] investigated potassium and calcium levels in blood tubes taken before and after kEDTA samples, showing that there was no significant difference between the samples. They did, however, exclude one patient due to difficult venipuncture and acknowledged that blood collection under less than ideal circumstances could cause inaccuracies in test results. This may be because syringe needles are more commonly used in difficult venipunctures or due to under-filling and droplet transfer. It, however, may also be due to potassium release from cells and concomitant dilution effect on calcium if there is local tissue damage during blood collection. In more recent studies, Salvagno et al. explored a wider range of biochemical parameters (potassium, sodium, calcium, magnesium and phosphate) in tubes taken before and after both kEDTA tubes and sodium citrate tubes. They clearly showed that there was no effect on any of the biochemical markers analysed [13]. Studies by Cornes et al. went one step further and analysed EDTA itself, alongside

**Table 1:** Recommended order of blood draw.

1. Blood culture tube
2. Coagulation tube
3. Serum tube with or without clot activators, with or without gel
4. Heparin tubes with or without gel
5. EDTA tubes
6. Glycolytic inhibitor tubes
7. Other tubes (e.g. trace elements)

other biochemical parameters (potassium, calcium, magnesium, zinc, ALP and iron) in samples taken before and after collection of EDTA blood using ideal closed-loop phlebotomy procedures for two different collection mechanisms [14, 15]. Their data agreed with that presented above in that incorrect order of draw under ideal phlebotomy conditions does not cause contamination irrespective of which closed blood collection system is used.

There are a couple of studies that have looked into how much EDTA is required to significantly affect biochemical parameters. Studies by Lima-Oliveira et al. and Cadamuro et al. looked at manually spiked samples with increasing amounts of EDTA to investigate how much EDTA is enough to cause erroneous results. Lima-Oliveira et al. showed that as little as a 5% sample contamination with EDTA was enough to affect calcium, magnesium, potassium, chloride and LDH, whereas other analytes like iron, phosphate and sodium required up to 30% contamination [16]. Cadamuro et al. performed a similar investigation, but focused their study on absolute volumes. They showed that magnesium was significantly affected following just 10  $\mu\text{L}$  of contamination followed shortly by potassium and calcium, which required just over 10  $\mu\text{L}$  to trigger their arbitrary  $\pm 10\%$  cut-off for significance. Iron was also affected but required  $>100 \mu\text{L}$  of contaminant [17]. These data are significant because the average size of a drop of liquid is 10–30  $\mu\text{L}$ , thus indicating that one single droplet of carryover would be enough to cause erroneous results.

Given the data presented above, the question remains as to whether recommending an order of blood draw is necessary. There are a number of case studies and investigations that show that sample contamination still occur indicating a need for a standardised and robust venesection procedure. Cornes et al. reported a case where a very high sodium measurement resulted in a patient being brought to the emergency department for further investigation [18]. On repeat, the sodium was normal, and upon questioning, the patient recalled the blood sample being poured from one bottle to another. Further investigation showed that in these cases, a direct ISE would be lower than indirect, the chloride would be inappropriately low and there would be a significant negative osmolar gap. Although not strictly due to order of draw, this highlights the errors that can occur if the venesection procedure is not well planned. Lima-Oliveira et al. published a case highlighting that incorrect order of draw does not just affect the main laboratory analysers. They presented a case where a patient had erroneous potassium and calcium results on a blood gas analyser due to EDTA contamination of an arterial blood gas syringe [19]. Data from Tunisia on rates of EDTA contamination in hyperkalaemic

samples (based on surrogate markers) before and after an awareness campaign showed a significant problem with EDTA contamination. The awareness campaign dropped the rate of contamination from 44.4% to 27% [20]. To definitively show how common EDTA contamination may really be, Cornes et al. performed a couple of studies. The first measured EDTA levels in all hyperkalaemic samples and the second measured EDTA in all hypomagnesaemic, hypocalcaemic and hypozincaemic samples over a 1 month period [21, 22]. The results proved that EDTA contamination is more common than expected and not always easy to identify (28 of 117 hyperkalaemic samples had a significant degree of EDTA contamination). Of greater concern, they showed that a significant number of contaminated samples were normocalcaemic, normomagnesaemic and normokalaemic. In one sample, the EDTA contamination masked a true hypokalaemia identified on subsequent samples, delaying the diagnosis. They also highlighted that a significant proportion of these samples were not identified by their current laboratory practice and recommended introducing EDTA analysis as a routine. Cornes et al. went on to investigate all hyperkalaemic samples from five different hospitals covering three different tube manufacturers [23]. The results show that EDTA contamination is not specific to site or tube type and that using surrogate markers alone misses some contaminated samples.

## Discussion

The data presented above have a few very clear messages. Firstly, bad practice in venous blood collection occurs and highlights a need for clear, robust and standardised guidelines. Secondly, if a closed-loop blood collection system is used, following the manufacturer's instructions, then order of draw is seemingly not important. Thirdly, and most importantly, there is very clear evidence that a failure to follow order of draw still occurs and may cause erroneous results, many of which may be missed in routine laboratory practice.

There are three possible mechanisms of contamination. The first, direct transfer, is easily identified and down to bad practice rather than order of draw, and the second, backflow, appears not to be the case, as when ideal phlebotomy conditions are in place, there is no effect of incorrect order of draw. The third possibility is contamination by syringe needle transfer. This occurs when blood is collected via needle and syringe and then added to tubes. This may increase the risk of blood droplet or additive transfer, which as shown above can be enough to cause

erroneous results. It is likely that carryover of a single droplet from a tube filled to the manufacturers recommendations would be insufficient to cause a significant interference, but transfer from poorly filled tubes (often seen in difficult venipuncture where syringes are more likely to be used) or needle contamination with additive could be enough to explain the contamination seen.

Decanting of samples from one tube to another is easy to spot in the laboratory. However, only a small amount of contamination is enough to cause erroneous results, and these small contaminations can be difficult to identify. In an observational exercise in a UK emergency department, 52% of samples were taken via an open-syringe method rather than the closed-loop recommended approach [24]. It was also noted that the EDTA tube was filled first in 41% of blood draws. Also, in a recent European observational study of venous blood collection, a failure to follow the order of draw was noticed in 8.1% of venous blood collections [25]. It is also important to note that it is not just laboratory professionals who are trained to know about and look for potential interferences that may need to interpret erroneous results. As reported in the case study above, contamination also affects electrolyte results when performed on blood gas analysers which may be misinterpreted by clinicians who are not aware of the risk, and this can have a significant patient risk if the erroneous results are acted upon.

Given the evidence presented here that a significant frequency of undetected sample contamination does occur regardless of the collection system used, the knowledge that closed-loop ideal phlebotomy conditions and protocols are frequently not followed or possible, and finally given that order of draw is not difficult to follow, we are in support that the recommendation to use the order of draw should be reiterated.

## Recommendation

Being an easy to implement and well-established practise, WG-PRE recommends that the order of draw for venous blood collection should continue to always be followed.

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