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Pre-Analytical Within-Laboratory Evacuated Blood-Collection Tube Quality Evaluation

Nataša Gros

Abstract

Pre-analytical steps contribute to an overall quality of the results of laboratory trials. The volume of blood drawn into the blood-collection tube and the anticoagulant amount introduced into the tube during its production should ensure the anticoagulant level in the recommended range; otherwise, the results can be altered. In evacuated blood-collection tubes, the internal under-pressure at the instant of the blood specimen collection affects the draw volume. During the shelf life, the internal under-pressure deteriorates. With no testing procedures in place, inappropriate anticoagulant levels can pass unnoticed. The chapter details testing procedures ensuring that the tubes are used only if, and only until, they are of the adequate quality. The reasoning behind the methodology is fully explained, and the case studies of the quality evaluations are discussed.

Keywords: evacuated blood-collection tubes, K_3EDTA , K_2EDTA , citrate, quality evaluation, draw-volume measurement, anticoagulant concentration

1. Introduction

Evacuated blood-collection tubes are evacuated containers intended for a venous blood specimen collection. They consist of a tube and a closure, which has to be tight to restrain low pressure—vacuum inside the tubes during their shelf life—but, on the other hand, it also has to be soft enough to let a sharp end of a blood-collection device to penetrate into a tube. The collection device has a disposable needle attached to the other side for phlebotomy.

Evacuated blood-collection tubes improved patients' and medical personnel's safety and mostly replaced classical tubes, which required a syringe and a needle for a specimen collection. In a continuation, wherever a tube is mentioned, the evacuated blood-collection tube is meant.

To prevent blood coagulation, tubes contain anticoagulants, either as dry substances attached to the internal walls or as solutions. Widely known and used are sodium citrate and salts of ethylenediaminetetraacetic acid (EDTA), usually present either as dipotassium or tripotassium salts, K_2EDTA or K_3EDTA .

Other substances, additives might be introduced as well to ensure the adequate properties or behavior of the tubes' internal walls or closures; nevertheless, they are expected not to interfere with a determination and affect analytical results. A noncompliant constituent detected in citrate tubes was magnesium which leached

from a stopper and was consequently influencing the prothrombin time (PT) results [1]. A comprehensive study evaluating different tubes comprising also recently introduced low-magnesium version confirmed that the PT and INR differences between the tubes are correlated with the magnesium concentration differences [2].

Manufacturers are obliged to specify on the label of a tube: a type of anticoagulant, a nominal draw volume, a lot number, and expiration date; within this text, we use a term expiry date as well.

Anticoagulant concentration in a blood sample after specimen collection should be within an appropriate range; otherwise, analytical results might be altered. To reach this objective, an accurate amount of anticoagulant should be introduced into a tube during production, and a draw at the moment of a specimen collection should be adequate to ensure a volume of blood entering a tube is within an acceptable range. A label on a tube provides guidance for inspection if the volume is within the suggested limits; however, this is only true if the label is precisely and accurately positioned.

The latest version of the GP39-A6 standard of the CLSI standardization body (Clinical and Laboratory Standards Institute) [3] requires of the tubes' manufacturers to ensure that until expiration date, the anticoagulant concentration remains within the 5% range of the value stated on a label. A draw volume is considered acceptable if it does not differ from the stated nominal volume for more than $\pm 10\%$.

The standard GP34-A recognizes the importance of appropriate blood-to-EDTA ratio for obtaining optimal examination results but avoids stating the exact limits. The EDTA can, if in a concentration which is too high hypertonicity shrink red cells, affect red cell size and cause morphological changes. On the other hand, it can too extensively chelate calcium and other cations such as magnesium and zinc and affect the activity of alkaline phosphatase enzyme label used in chemiluminescent assays or reduce the efficiency of the recognition of proteins by antibodies due to the proteins' conformation changes [4].

The predecessor of CLSI, the National Committee for Clinical Laboratory Standards (NCLLS), was in the H1-A5 standard more explicit in terms of some anticoagulants' concentrations [5]. It explains that only a little bit less than a half (1.15 mmol/L) out of the total calcium concentration (2.5 mmol/L) corresponds to unbound calcium that needs to be chelated stoichiometrically with EDTA to prevent coagulation. For that reason, it suggests that EDTA concentration in blood should be between 3.7 and 5.4 mmol/L, since excessive concentration causes morphological changes in the blood.

Not consistent with this requirement was DIN ISO 6710: 1996-12 standard requiring the EDTA concentration within the 4.11–6.843 mmol/L range [6].

A potential user can during time come across the tubes which were produced by not having the same set of requirements on the mind. As we already previously demonstrated, the tubes if evaluated as such not yet in contact with a blood sample are not all the same, and change in their own characteristics during their shelf life and the testing procedure which we suggested are easy to perform [7].

A concise review reflects on the behavior of EDTA as an anticoagulant in hematology and furthermore discusses its usage in proteomics, general clinical chemistry, and its applicability for measuring cytokines, protein, peptides, and cardiac markers [8]. Elsewhere, influences of a form of EDTA and its concentration on the results of hematological tests were profoundly discussed in relation to spurious counts and results regarding platelets [9], white blood cells, red blood cells, hemoglobin, red cell indices, and reticulocytes [10]; under-filled or over-filled evacuated tubes changing the anticoagulant level in a sample are exposed as an influential pre-analytical source of errors.

For citrate tubes the DIN ISO 6710: 1996-12 standard recommends trisodium citrate solutions with concentrations between 100 and 136 mmol/L; however, the H1-A5 standard specifies the concentrations 105, 109, and 129 mmol/L.

Due to all these differences, the GP39-A6 standard omitted all the anticoagulant concentrations' details, leaving it entirely to a producer to bear the responsibility for securing appropriate concentration, fulfilling all the requirements, and demonstrating that they are actually met, or in other words verifying that the tubes are actually fit for purpose.

The GP34-A standard provides guidance for validation and verification of tubes for venous and capillary blood specimen collection [4]. Both a manufacturer and a clinical laboratory are required to perform a comparability study on blood samples for two or more sets of tubes comprising a set which was already evaluated and approved previously. A manufacturer performs such a test after a new product was developed or where any correction actions are necessary for the production process. The laboratory needs to do it when switching from one product to another or when changing a vendor.

A within-tube precision study requires a minimum of 20 subjects, and each sample needs to be analyzed in replicates; an appropriate number of samples, evenly distributed through the analytical measurement, are essential for trueness evaluation [4]. Several studies with sometimes dissimilar outcomes can be found in the literature.

Two blood-collection devices either with an aligned [Becton Dickinson (BD)] or at an angle needle holder (Greiner Labortechnik GmbH) were evaluated either enabling a direct linear (BD) or interrupted nonlinear blood flow. A mechanical strain on blood cells was recognized as a factor potentially causing the efflux of intracellular constituents into the serum in an interrupted nonlinear flow. The magnesium, plasma hemoglobin, and prothrombin time within-subject variations were confirmed in 55 healthy individuals using a Student paired t-test. A difference in a tube material either glass or polymer was also recognized as a likely contributing factor [11].

Nevertheless, contrasting outcomes were obtained for prothrombin time determinations in the glass and PVC tubes with two distinct citrate concentrations where neither material caused the significantly different results [12]. Yet another study, establishing a protocol for comparing the citrate evacuation blood-collection tubes with glass tubes employing eight measuring systems, confirmed a statistically significant but clinically not relevant difference in prothrombin time results, which were more pronounced with the tubes of the lowest 2.7 mL draw volume [13].

Differences in some parameters were confirmed if BD plastic citrate tubes were used instead of glass tubes, but they were considered unlikely to be clinically significant [14], though a comprehensiveness of a study was challenged arguing that only healthy volunteers were involved and by these means it was not yet proven that glass tubes are interchangeable with the plastic tubes [15]. But a study performed on Greiner glass citrate and plastic tubes confirmed that the tubes are substitutable as far as either untreated or patients on a traditional oral anticoagulant therapy are concerned and that this applies for the whole shelf life of the tubes [16].

Nevertheless, the plastic tubes of different brands evaluated on patients and healthy volunteers were confirmed to be statistically but not clinically significantly different [17]. For patients on oral anticoagulant therapy with vitamin K antagonists, ANOVA test confirmed statistically significant differences in prothrombin time for the tubes of four different types [18]. The study supports the claim that validation is always necessary when there is a change in a tube type.

A research performed on a group of individuals evaluating the effect of under-filled EDTA tubes on hematological parameters by employing a particular type of

analyzer [19] leads to contrasting outcomes not necessarily aligned with other studies [20] and general principles and recommendations.

Validations and verifications as required by the standard GP34-A are complex to perform, time demanding, and require resourceful personnel [4].

The standard exposes a blood collection as a pre-analytical (preexamination) source causing varying degrees of errors. It brings to light a lack of a mechanism that would enable systematic evaluations of the influences of pre-analytical (preexamination) variables on laboratory test (examination) results [4].

The characteristics of the tubes entering the pre-analytical phase are such variables, and this is where this chapter tends to contribute.

Differences between tubes of different brands examined 5 years apart in time are going to be enlightened, and the testing procedures which are fast, cheap, and easy to implement into laboratory practice are explained in full details. Robustness of personal profiles of athletes and validation studies performed on blood samples can profit from knowing the attributes of the tubes that were actually used or evaluated.

2. Quality of evacuated blood-collection tubes for hematological tests reevaluated in 5 years' time

In this section, we compare the results of a quality evaluation of the blood-collection tubes, on which we previously reported [7], with the results obtained in 5 years' time. The same producers were considered, namely, Becton Dickinson, Greiner Bio-One, and Laboratorijska tehnika Burnik d. o. o.

Figures 1–4 are dedicated to the tubes of four different brands. The abscise axis stands for a draw volume. Ordinate on the right indicates an anticoagulant concentration expected for a blood sample after a specimen collection. We are going to explain the meaning of the left ordinate axis, which relates to a testing procedure, later. A frame in green indicates the limits set by the H1-A5 standard [5]. The horizontal lines confine the range of the acceptable anticoagulant concentration; the left vertical line represents a limit under which a draw volume is expected not to fall to prevent the anticoagulant concentration to rising too high.

We kept the assigned marks A, B, and C from the previous study for the K_3EDTA tubes, and D for the K_2EDTA tubes, not disclosing the producers' identity. We additionally included the tubes F, not previously evaluated. The more recent

Figure 1. Anticoagulant concentrations and draw volumes of the A brand tubes obtained 5 years apart in time (orange/blue) and of the tubes F not previously included (the numbers in the labels indicate time until the expiration).

Figure 2.
Anticoagulant concentrations and draw volumes of the B brand tubes obtained 5 years apart in time (orange/blue); the numbers in the labels indicate the time until the expiration.

Figure 3.
Anticoagulant concentrations and draw volumes of the C brand tubes obtained 5 years apart in time (orange/blue); the numbers in the labels indicate the time until the expiration.

Figure 4.
Anticoagulant concentrations and draw volumes of the D brand tubes obtained 5 years apart in time (orange/blue); the numbers in the labels indicate the time until the expiration.

results are marked with an asterisk; a number indicates the number of days until the expiration.

Ellipses in **Figures 1–4** provide an insight into a spread of results obtained for the tubes within a series of measurements. Ellipses in blue correspond to the more recent results; those in orange originate from a previous study. A length of a horizontal axis of an ellipse equals a standard deviation of the draw-volume measurements; a vertical axis indicates a standard deviation of the anticoagulant concentration as expected for blood samples. A crossing of the two axes of an ellipse is defined by the mean values of the two parameters. The smaller the ellipse, the higher the quality of the produced tubes in terms of their precision or, in other words, a repeatability of a product.

2.1 K₃EDTA tubes

From this point of view, the A brand tubes were and remain of a high quality. In spite of having a very long expiration date, unless very close to the expiration (A*18), the draw volumes generally remain adequate; however, the anticoagulant concentration is too high and not in the accordance with the higher limit set by the H1-A5 standard [5]. All the ellipses of the A brand tubes are above the green rectangle in **Figure 1**. The same is true for the tubes F, which are of the same manufacturer. This example demonstrates that an adequate draw volume does not yet ensure the adequate anticoagulant concentration as far as the H1-A5 is concerned. The tubes were obviously produced following an older DIN ISO 6710: 1996-12 standard, for which the anticoagulant concentration 6.843 mmol/L was still acceptable. With this limit in mind, only the A*18A anticoagulant concentration would be excessive. The characteristics of these tubes clearly contrast those of the tubes B and C, as the figures in the continuation demonstrate.

In the B brand tubes (**Figure 2**), an improvement in the overall quality of the results is evident in 5 years' time. Previously the ellipses were much larger, and they exceeded the upper anticoagulant concentration limit even with the draw volumes very close to the nominal 3 mL mark. The ellipses in blue are small and remain within the green rectangle during the whole shelf life; nevertheless it has to be mentioned the shelf life was at the time of purchase much shorter than in the A brand tubes.

The C brand tubes exhibit a good repeatability of the product; however, the anticoagulant concentration starts exceeding slightly the higher-anticoagulant concentration limit already with the draw volumes approaching 2.8 mL, proving that the draw volume within the acceptable range does not yet guarantee the correct anticoagulant concentration.

2.2 K₂EDTA tubes

The product repeatability of the D brand tubes is good, but the anticoagulant concentration starts exceeding the upper limit already approximately 200 days before the expiration date and with the draw volumes falling below 2.9 mL what is far above the acceptable lower limit.

This study confirmed that a control of a draw volume is not the main quality issue and does not ensure that the anticoagulant concentration is adequate. Only in four cases, the draw volumes below the 2.7 mL limit were observed, and this only happened when the tubes were tested closer than 20 days before their expiration date. On the other hand, in more than 20 cases, the anticoagulant concentration limit as set by the H1-A5 standard was exceeded at the draw volumes in the recommended range.

It also needs to be mentioned that if the draw-volume inspection relies on the label mark a judgment can be false. During our first study, we found out that only one brand of the tubes had a label positioned precisely; in all others the indicators on the tube were misleading.

Easy-to-perform testing procedures as we used here which do not require blood samples can as a precautionary measure ensure that the tubes are used only if they are of the adequate quality and their quality does not fluctuate too much during the time. It can alert a laboratory when it would be advisable to perform a much more complex and time-demanding verification study on blood samples. Archived data on the tubes' characteristics and quality during a longer period of time can provide a piece of evidence for other studies and rule the tubes out as a potential cause for variations.

2.3 Tubes' drawing capability reduces during the time

In this section, we explain the changes in a behavior of the tubes during their shelf life.

The tubes' drawing capability depends on a difference between the external (p_{st}) and internal ($p_{int_20^{\circ}C}$) pressure. The lower the internal pressure, and the higher the difference to the external pressure, the higher a drawing capability. A tube's internal volume (V_{tube}) also contributes to higher capacity to draw a liquid.

The tubes of different brands differ in their drawing capability and, in a way, how it reduces during the time, as **Figure 5** demonstrates. No container is entirely tight and leaks to some extent. The conditions to which the tubes were exposed or under which they were stored contribute. The tubes of the same lot would behave differently in different circumstances.

Even though tubes are of a high quality, are purchased at the same time, and are of the same lot, they are not all the same if used during their shelf life.

Figure 5.
Drawing capability of the tubes reduces continuously during their shelf life.

A reduction in a drawing capacity results in a diminished draw volume and enhanced anticoagulant concentration.

The tubes' validations and verifications with blood samples are essential; however complex and with no insight into characteristics of the tubes as such, it is not clear what was actually tested and how representative it is. The outcomes might vary and can be influenced by a choice of a group of individuals, its representativeness, a normality of a distribution of the investigated parameter, and the sources of uncertainty originating from the whole procedure, comprising pre-analytical, analytical, and post-analytical phases. All these factors can influence the conclusions of a paired t-test or ANOVA, a difference between two brands, or lots of the tubes might turn out insignificant. Insight into tubes' characteristics can give the verification and validation studies some basic orientation on what was the status of the tubes that were investigated. If the evaluation is repeated later, one can know how comparable are the examined tubes entering the process.

If a difference between the results obtained for the tubes of different brands is confirmed to be statistically significant by the tests performed on blood samples, they are frequently considered not clinically important. However, variations, which are not important on the level of a group of individuals, might reflect differently when a single person is concerned. Personal variations in blood parameters are narrower than variations on the level of a population. A personal medicine and athlete's biological passport require higher sensitivity and attention paid to all sources of uncertainty.

It is not possible to perform validation study with blood samples for each individual, but it is easily possible to perform a quality control of the tubes which are used for personal profiles.

The athlete's biological passport (ABP) requires accurate and reliable results of hematological tests, which are stable enough for evaluations of the probabilities of abnormalities in a personal profile. Hence, some parameters, e.g., hemoglobin and erythrocytes, were identified as highly stable; the others such as reticulocytes, mean red blood cell volume, and hematocrit did not turn out as such. Sample storage conditions and treatment and the choice of an analyzer are considered the contributing factors [21].

It was proven that during a training season hemoglobin and hematocrit reduce in their value, and reticulocytes do as well but independently. The pattern is general but the size of a change is sport's discipline dependent. It was recognized that reliable reference ranges in sportsmen could not be defined without the best laboratory practices [22].

Not univocal and entirely clear outcomes of different studies on the stability of the blood variables raise concerns and request for more clearly defined characteristics, procedures, threshold limits, personal reference ranges, and criteria for recognizing abnormalities to prevent false convictions in athletes [23].

In order to raise awareness to which extent different pre-analytical phases could affect the outcomes of hematological and biochemical tests on which sports medicine depends in following athletes, different pre-analytical aspects and the choice of anticoagulant, instabilities of some molecules were addressed to prevent misinterpretation of data and improve the usefulness of results [24]. Specimen homogenization as a pre-analytical phase received special attention [25].

The variations in tubes' characteristics did not receive attention in relation to ABP in spite of the fact that easy-to-perform testing procedures are available [7]. They are thoroughly explained in the continuation.

3. A methodology for K₃EDTA or K₂EDTA evacuated blood-collection tubes' quality evaluation

A methodology for a quality evaluation of evacuated blood-collection tubes for hematological tests consists of two successive measurements, a measurement of a draw volume and electrolytic conductivity, from which one can predict the anticoagulant concentration in a blood sample. No patient- or person-related samples are required. A medium for the tests is purified water.

Only low-cost equipment, a Bang burette and a field conductivity meter, is used. One also needs to know an ambient temperature and a non-reduced pressure for a period during which measurements were performed. The latter can be obtained from a local meteorological institution on request, and temperature is easy to measure. We explain the testing procedures in full details in the following section.

We previously published the nomograms for K₂EDTA and K₃EDTA tubes with nominal 3 mL draw volume, which enable a prediction on how is an anticoagulant concentration going to rise with a diminishing draw volume that happens during the time because of the aging of the tubes [7].

In **Figure 6** we present a nomogram for the K₃EDTA tubes with a 2 mL nominal draw volume relating the electrolytic conductivity, κ , with a predicted anticoagulant concentration, c , and a draw volume, V . The points are the results of the quality evaluation of the tubes of two different brands, E and G, at the particular moment in time. If the tubes in a lot are of a homogenous quality and they are going to be tested later during their shelf life, the anticoagulant concentration as expected for blood samples is going to rise as the curves indicate. In such a case, a draw-volume measurement can already give an insight into the quality of the examined tubes.

If we take the G tubes as an example, for the great majority of the tested tubes, with a draw volume close to 2.1 mL, the anticoagulant concentration anticipated initially was 4.45 mmol/L. If we assume that later in time we find out that the draw volume has fallen to 1.9 mL, the anticoagulant concentration for blood samples is going to be close to 4.95 mmol/L. A green rectangle indicates the limits set by H1-A5 standard [5], demonstrating that the tubes would still have been of adequate quality.

Skills needed to perform the tests are not difficult to master, and a laboratory or medical staff can easily develop them. The quality of the tubes does not deteriorate very rapidly. It is important to test tubes when they are put into use, and later only

Figure 6.
A nomogram for a prediction on how is the anticoagulant concentration in 2 mL K₃EDTA tubes of two different brands (E, G) going to change with a declining draw volume; green rectangle outlines the characteristics considered acceptable by H1-A5 standard [5].

occasionally, but at regular intervals. Since the tests are not time-consuming and not performed in high numbers, this additional workload does not represent an important additional burden for personnel; however, the benefits for an institution are obvious and important.

An institution implementing a quality evaluation scheme always has an adequate insight into the characteristics and quality of the tubes it is using. It can ensure that the tubes are used only if and only until they are of adequate quality or it can use the data in medical and clinical studies to test possible correlations or covariations.

4. Testing procedures

Testing procedures consist of a draw volume and an electrolytic conductivity measurement (**Figure 7** left/right), both later corrected to correspond to a temperature 20°C and an external pressure 101 kPa as required by the standards [5, 6].

4.1 Draw volume

In the schematic (**Figure 7**), far left, an evacuated tube, characterized by an internal pressure (p_{int}) and an internal tube volume (V_{tube}), defining a conserved energy of withdrawal for a blood specimen collection is depicted.

In the middle, a draw-volume measurement is schematically represented. A starting point is a Bang burette filled with purified water to the 0 mL mark. A tip of the burette is attached to a flexible tubing, which is at the other end connected to a blood-collection device. This part too is entirely filled with purified water. When we attach an evacuated tube to the venipuncture device, the tube starts filling with water, and consequently the water level in the Bang burette starts falling. The rising water level in the tube acts as a moving piston, reducing the void volume and causing the internal pressure to rise until it equals the external pressure (p_{ext}). At this moment, the withdrawing ends, and we can read the draw volume from a burette.

Hence, the external pressure and temperature (T) affect a draw-volume test. The external pressure depends on the altitude and current weather conditions and can be obtained from the local meteorological institution. The ambient temperature is also important and has to be taken into account. It affects the internal pressure in

Figure 7.
A setup for a draw-volume measurement (left) and a conductivity cell for the anticoagulant concentration estimation (right).

a tube at the instant of the draw-volume measurement. At a higher temperature, the air in the tube expands, the internal pressure rises, or, in other words, the internal under-pressure deteriorates, and a tube's withdrawing ability falls.

For these two reasons, a draw volume (V_{draw}) should be corrected to obtain an estimation of a draw volume, as it would have been, if measured at 1013 hPa and 20°C ($V_{\text{draw_st}}$) (1); the symbol K and hPa stand for the units Kelvin and hectopascal, respectively:

$$V_{\text{draw_st}} = V_{\text{tube}} - \frac{p_{\text{ext}} \times 293.16 \times K}{T \times 1013 \times \text{hPa}} (V_{\text{tube}} - V_{\text{draw}}) \quad (1)$$

The standards [5, 6] admit that if the draw volume under these conditions does not differ from the nominal volume for more than 10%, the tubes are expected to be of the adequate quality for a blood specimen collection. For the 3 mL tubes, this means that the draw volumes between 2.7 and 3.3 mL are acceptable.

Even though the standards [3, 4] expose as the main quality issue in a nonconformity of a draw volume with the requirements, our results in Section 2 prove that a draw volume within the acceptable range does not necessarily ensure the correct anticoagulant level. An additional insight into this aspect of quality is necessary. For the K₂EDTA and K₃EDTA tubes, respectively, a conductance measurement can provide such an insight.

4.2 Ionic conductivity

K₂EDTA and K₃EDTA are salts, and salt in water dissociates in ions. Solutions containing ions conduct electric current, to which extent depends on the ions' characteristics and their concentration. In other words, conductance (G) of a solution reflects an overall ionic composition, but, if a solution contains only a single salt, as it is a case for K₂EDTA and K₃EDTA tubes, it can provide an insight into a salt concentration.

A conductance depends on the geometry of a conductivity cell. A cell we used is depicted in **Figure 7** (far right). It was an immersive four-electrode cell, not directly applicable to our needs. We closed the bottom of the cave with a parafilm and the hole at the right with a stopper made of a pipette tip to transform it into a conductivity cell applicable for conductance measurements in solutions of a small volume.

The conductance measurement is affected by a size of electrodes and a distance between them; a cell geometry is reflected in a cell constant K . If the results of measurements are expressed as ionic conductivity (κ), they are universally useful and comparable between different measuring systems (2):

$$\kappa = G \times K \quad (2)$$

Another concern is a temperature during a measurement; it also affects the conductance value. At higher temperatures, a conductance is higher. This is taken into account by reporting an ionic conductivity at a selected reference temperature, e.g., 20°C. Measurements obtained at an ambient temperature are transformed into the values as would have been at the reference temperature by taking a temperature compensation into account. A linear compensation is frequently used; a compensation factor is usually approximately 2%/°C. In our case, the values confirmed experimentally were 1.99 or 2.01% for K₃EDTA or K₂EDTA, respectively.

Conductivity measurements are in fact easy to perform. A conductivity cell has a temperature sensor incorporated. We select the reference temperature, define a temperature compensation factor and a cell constant, and perform measurements.

4.3 Anticoagulant concentration

For a solution of a single salt, electrolytic conductivity values are easy to transform into a salt amount concentration, c . If one prepares a set of the solutions with the known salt concentrations and determines their electrolytic conductivity at the reference temperature, one can relate the two parameters by depicting a graph, with the first parameter on the abscise axis, and the second on the ordinate, defining a trend line (3):

$$\kappa = a + b \times c \quad (3)$$

The symbols, a and b , are the parameter of the linear equation; a stands for the intercept and b for the slope.

After these two parameters are known, one can measure the electrolytic conductivity of the anticoagulant solution obtained after a draw-volume test to obtain κ_{V_draw} and use the equation in a rearranged form to calculate a concentration of the anticoagulant, c_{V_draw} (4):

$$c_{V_draw} = \frac{\kappa_{V_draw} - a}{b} \quad (4)$$

This concentration is valid at the draw volume, but one wants to know the anticoagulant concentration as expected in blood after a specimen collection, $c_{V_draw_st}$. As already explained, the draw volume had to be corrected to obtain an estimation of a blood sample volume; a concentration is volume dependent, and therefore it has to be corrected too, to correctly represent the anticoagulant concentration as expected after a blood specimen collection (5):

$$c_{V_draw_st} = c_{V_draw} \frac{V_{draw}}{V_{draw_st}} \quad (5)$$

Draw volume and anticoagulant concentration determination can be implemented into laboratory quality control routines. If followed during the time in a form of control charts, they can ensure that the tubes are used only if and only until they are of adequate quality.

5. Citrate tubes a distinct case

The same testing procedure for a draw-volume measurement is applicable also to citrate tubes. However, in terms of a determination of the anticoagulant concentration, this is a distinct case. The reason is that citrate in the tubes can be either buffered or unbuffered. As a result, a solution after a draw-volume test can have quite a different pH and contains different citrate equilibrium forms in different proportions. For this reason measurement of electrolytic conductivity cannot be used here, and an adequate low-cost and easy-to-perform testing procedure yet has to be developed.

We evaluated the citrate tubes of two different brands, A and B. Both were (1:9) type tubes but differed in nominal draw volumes, which were 4.5 and 3.6 mL for tubes A and B, respectively. Measured draw volumes that we obtained were 4.55 and 3.47 mL; it has to be pointed out that these are uncorrected values.

Getting insight into the composition of the anticoagulant solution after a draw-volume test is possible, but the approach is complex and time-consuming. We used

Figure 8.
 Chromatograms for determination of sodium, potassium, magnesium, and calcium in the tubes of two different brands, A and B, after a draw-volume test.

two ion chromatographic techniques, ion-exclusion chromatography for determination of citrate and ion-exchange chromatography for determination of cations.

Figure 8 depicts chromatograms obtained for determinations of cations after a draw-volume test. Four chromatographic peaks emerge. The first peak appearing between 4 and 6 minutes pertains to the major cation, sodium, originating from the anticoagulant, trisodium citrate. This peak is presented twice with two different y-axis scales, in the main chromatogram with its top is out of scale and in the inserted frame where it is fully visible.

The peak for the A brand tubes is smaller, indicating that in this case citrate is buffered, while in addition to sodium ions there are hydronium ions (H^+ , or H_3O^+) to neutralize a negative charge of citrate equilibrium forms. In other words, in the case of the A brand tubes, the anticoagulant solution was prepared not only from a trisodium citrate but also with an addition of citric acid. Consequently, the pH of a solution is lower than with the B brand tubes; the pH results we obtained were 6.1 and 7.9, correspondingly.

As **Figure 8** demonstrates, we also confirmed the presence of potassium, a peak between 6 and 8 minutes; magnesium, a peak at around 10 minutes; and calcium, a peak at around 12 minutes.

Presence of magnesium came with no surprise; authors using a different analytical method previously reported on it and explained that it leaks from a closure and originates from an additive [1]. Though we confirmed a difference between A and B brand tubes, the latter contained magnesium in much lower concentration.

The concentration of calcium is low in both cases and might be considered an impurity originating from other chemicals. But what makes a real difference between the tubes of the two brands and was not expected is potassium. While it is nearly inexistent in tube B, it is obviously present in tube A. We used infrared spectroscopy to explain where it originates from; it appears that the source might be a tripotassium salt of EDTA; if this is the case, its concentration is approximately 200 times lower than the citrate concentration.

6. Conclusions

This research proved differences in characteristics of tubes of different brands and different lots, and their attributes are also changing during a shelf life. We suggested fast, easy-to-perform testing procedures, which already by using purified

water and low-cost equipment only give an insight into draw volume and anticoagulant concentration as can be expected for a blood specimen collection.

No doubt, a laboratory has to prove on blood samples that a particular type of tubes it is using or intends to use is fit for purpose. But already with a minimum required number of samples of 20 individuals, a study becomes complex, professionally demanding, and time-consuming. However, not knowing the characteristics of the tubes entering the investigation lacks generality.

Testing procedures as suggested are not to replace but to support such investigations and to make them more economical. A quality control of the tubes as such is easy to introduce into a laboratory practice and does not importantly contribute to a workload. The tubes do not change very rapidly over the time and do not need to be tested very frequently if of the same lot. However, insight into their characteristics provides guidance when a study on blood samples needs to step in to cover important distinctive conditions a laboratory is likely to face during its everyday routine.

A draw-volume test we described is generally applicable. Procedures for determining K_2 EDTA and K_3 EDTA concentrations in the tubes before a specimen collection are well established; nevertheless comparably easy-to-perform testing procedures for citrate tubes yet need to be developed.

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Conflict of interest

No conflicts of interest are declared by the authors.

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