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Evaluation of small-volume tubes for venous and capillary PT (INR) samples

L. ONELÖV, R. BASMAJI, A. SVENSSON, M. NILSSON, J. P. ANTOVIC

Department of Coagulation Research, Institute for Molecular Medicine and Surgery, Karolinska Institutet & Department of Clinical Chemistry, Karolinska University Hospital, Stockholm, Sweden

Correspondence:
Jovan P. Antovic, Department of Molecular Medicine & Surgery, Karolinska Institutet, Consultant, Coagulation, Clinical Chemistry L704, Karolinska University Laboratory, Karolinska University Hospital, SE-171 76 Stockholm, Sweden.
Tel.: +46 8 517 75637 or +46 734 294447;
Fax: +46 8 517 76150;
E-mails: Jovan.Antovic@ki.se, Jovan.Antovic@karolinska.se

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SUMMARY

Introduction: Frequent PT (INR) testing may represent a problem for patients on warfarin treatment, and capillary or small-volume tubes may be more appropriate for such patients. A demand for small-volume tubes also comes from pediatric wards. Yet, while various small-volume tubes are available, they have not been properly evaluated.

Methods: Three small-volume tubes were tested (MiniCollect 3.8% citrate, MiniCollect 3.2% citrate and Microvette EDTA) and compared with a standard 4.5-mL 3.2% citrated tube. Samples were taken by venipuncture from the back of the hand and by capillary sampling from the tip of the finger. The measures were compared with those after standard venipuncture of the arm fold. A total of 180 samples, using different combinations of tubes and sampling sites, were collected from 30 volunteers.

Results: There were no differences in the results obtained using citrate tubes for venous samples in comparison with those obtained by standard sampling, while the results when using EDTA tubes were not comparable to those obtained by standard sampling ($P < 0.001$), expressing systematically lower values (by about 10%). The results observed after capillary sampling were significantly different to those obtained after standard sampling.

Conclusions: The MiniCollect 3.2% tube may be used for PT (INR) venipuncture samples when withdrawal of a small amount of blood is preferable, while EDTA tubes should not be used for PT (INR) testing.

INTRODUCTION

Prothrombin time (PT) together with the international normalized ratio (INR) is a measure used for determining the vitamin K-dependent factors in a blood sample. As such, it is important for monitoring treatment with vitamin K antagonists such as warfarin, while it is also used to monitor the protein synthesis capacity of the liver. Patients on warfarin treatment undergo sampling every 4–6 weeks (much more often at the beginning), and it would be beneficial to use small-volume sampling and/or sampling from sites

other than the arm fold, especially as some patients would prefer sampling from the tip of the finger. Small-volume sampling is also important for small children and neonates, from whom large volumes of blood cannot be drawn. There are already some published studies in which the authors compared different tubes in connection with coagulation testing [1–3]. However, no one has investigated what would happen if blood was withdrawn from sites other than the arm fold. One frequently used alternative for taking smaller samples is capillary sampling from the tip of the finger. However, capillary sampling is more difficult than venipuncture and only trained personnel can be entrusted to withdraw samples in this way [4]. In addition, the pre-analytical handling of the sample differs as regards venous and capillary samples, as capillary samples need to be diluted before they can be placed in the instrument. Even so, there are studies showing that PT results after capillary sampling can be compared with results after venous sampling [5–7].

Worldwide, two different methodologies for PT analysis are used – the Quick method [8, 9] and Owren's method [10]. In the Quick method, 1/3 undiluted citrated plasma is mixed with 1/3 thromboplastin and 1/3 calcium reagent, while in the Owren's method, citrated plasma is diluted 1/7 with buffer before it is mixed with 1/3 thromboplastin + fibrinogen + factor V and 1/3 calcium. In Owren's method, the plasma is hence more diluted and the sample constitutes a small part of the final volume. In Sweden and the rest of Scandinavia, Owren's method is most commonly used, where low interlaboratory variation has been described [11]. In addition, Owren's method seems to work better for a variety of different sample types and it has a precise, reproducible, and accurate calibration procedure [12]. Issues of sampling may be of importance even if Owren's reagent and significant predilution of the samples are used.

With the aim of investigating whether small-volume tubes may be used as an alternative to the standard 4.5-mL 3.2% citrate tube currently used for venous samples, three small-volume tubes with citrate or EDTA were evaluated. PT (INR) results in connection with both venous and capillary sampling were compared with those obtained by the standard sampling procedure recommended by the Clinical Chemistry Department at Karolinska University Laboratory. In order not to increase the sampling load for patients on warfarin, we turned to volunteers for blood samples for this study.

MATERIALS AND METHODS

A total of 180 blood samples were obtained from thirty volunteers, who all gave their informed consent to be included in the study. As all samples were compared on an individual basis, no specific exclusion criteria were used. One person was on warfarin treatment and therefore excluded from the final evaluation (174 samples were evaluated). All sampling procedures were performed by trained personnel at Karolinska University laboratory in Huddinge, or at Söder Hospital, in accordance with local guidelines. Analysis of PT (INR) according to Owren's methodology was performed in compliance with standard operating procedures for venous and capillary samples at the coagulation laboratory, Department of Clinical Chemistry, Karolinska University Hospital, using a Sysmex CS-2100i system (Siemens, Marburg, Germany) with SPA+ reagents from Stago (Asnières sur Seine Cedex, France).

Four different tubes for venous or capillary blood samples were used and compared in this study. The 4.5-mL Na citrate tube 0.105M/3.2% (Becton Dickinson, Franklin Lakes, US) is the recommended tube for routine coagulation assays at Karolinska University Laboratory and is referred to as the reference tube in this study. This tube was used for blood samples obtained via venipuncture at the arm fold. The MiniCollect 1-mL 9NC Coag 3.2% and the MiniCollect 1-mL 9NC Coag 3.8% from Greiner Bio-One (Kremsmünster, Austria) as well as the Microvette 500 (EDTA) from Sarstedt (Nümbrecht, Germany) were originally recommended as capillary blood collection tubes. However, in this study, they were tested in connection with both types of blood samples: venous (from the back of the hand) and capillary (from the finger tip). The tubes and sites of sampling that were compared in this study are listed in Table 1.

Two-sided paired Student's *t*-tests were performed (Microsoft Office Excel 2007) to find any possible statistically significant differences in the results. Probability values (p-values) below 0.05 were considered significant. The Bland–Altman test (Graph Pad 5.04 for Windows; Graph Pad Software Inc., La Jolla, CA, USA) was used to identify trends and visualize the magnitude

Table 1. Combinations of tubes and withdrawal sites. Venous sampling was from both the arm fold and the back of the hand, while all instances of capillary sampling were from the finger tip

	Citrated 5 mL, 0.105 M/3.2%	MiniCollect 1 mL 3.8%	MiniCollect 1 mL 3.2%	Microvette 500 (EDTA)
Arm fold	Venipuncture	Not done	Not done	Not done
Back of the hand	Not done	Venipuncture	Venipuncture	Venipuncture
Fingertip	Not done	Not done	Capillary	Capillary

of disagreement between the results. An equal distribution around zero was considered the optimal result, while one-sided distribution on either side of zero was considered to be a sign of an unacceptable trend.

RESULTS

Results for individual samples are shown in Table S1. The between-sample values for each volunteer differed by ±5% for all combinations of citrated tubes, with a good distribution around zero as seen in the Bland–Altman diagrams in Figure 1a,b and c and a correlation of at least 0.95 for all three tube/sampling site combinations (MiniCollect 3.8% venous sampling, $y = 0.9595x + 0.043$; MiniCollect 3.2% venous sampling, $y = 0.9573x - 0.0438$; MiniCollect 3.2% capillary sampling, $y = 1.0384x - 0.0311$). There was no difference in the results obtained with venous samples in citrated tubes in comparison with the reference tubes ($P = 0.48$ and $P = 0.82$ for 3.8% and 3.2%, respectively). However, the results obtained with capillary samples in the 3.2% citrated tubes showed a significant difference ($P = 0.04$) in comparison with the reference tubes. Regarding the EDTA Microvette tubes, correlation to the reference tubes was low at 0.8699 (venous sampling, $y = 0.9424x + 0.0206$) and 0.66 (capillary sampling, $y = 0.8767x - 0.1593$). In addition, the majority of values were lower than those in connection with the reference tubes, irrespective of the sampling technique. The results after venipuncture (with EDTA tubes) were up to 8% lower (Figure 2a), while the results after capillary sampling were up to 10% lower (Figure 2b) than the reference tube results.

DISCUSSION

The aim of this study was to see whether small-volume tubes may be used as an alternative to the standard 4.5-mL 3.2% citrate tubes used for PT (INR) analysis at Karolinska University Laboratory. A smaller-volume tube would make the sampling procedure easier both for adults on warfarin treatment and, in particular, for small children and neonates undergoing PT (INR) testing. Therefore, three different tubes were tested: the MiniCollect 1-mL 9NC Coag 3.2%, the MiniCollect 1-mL 9NC Coag 3.8%, and the Microvette 500. They were evaluated in connection with both capillary and venous samples. The PT (INR) results obtained when using both MiniCollect tubes for venous samples were not different from the results obtained after using the standard tubes ($P = 0.48$ and $P = 0.82$) and they may therefore be used as potential alternatives for blood samples when a smaller amount of blood should be taken. However, when these tubes were used for capillary samples, a difference ($P = 0.04$) in results in comparison with the reference tubes was observed. This difference might, however, be acceptable in specific cases, as it was only around 5% and the majority of the samples gave slightly higher results *vs.* the reference tubes. Therefore, most probably such a difference would not influence therapeutic decisions. Reports of CV_W (within subject variation) among healthy individuals of 2.3% [13] and 5.8% [14], and in individuals on anticoagulation therapy a CV_W of 9% [15] suggest that the 5% difference observed in this study may still be acceptable, particularly as the acceptable CV for PT (INR) runs at Karolinska University Hospital is set at 5% for normal INR values and 6% for therapeutic INR values. However, we have to emphasize that the observed CV is usually even lower (<4%).

The role of citrate and EDTA in the collection tubes is to prevent clot formation in the tubes by removal of Ca^{2+}. EDTA tubes are more commonly used for hematological testing [16], and different investigators have reached different conclusions in terms of

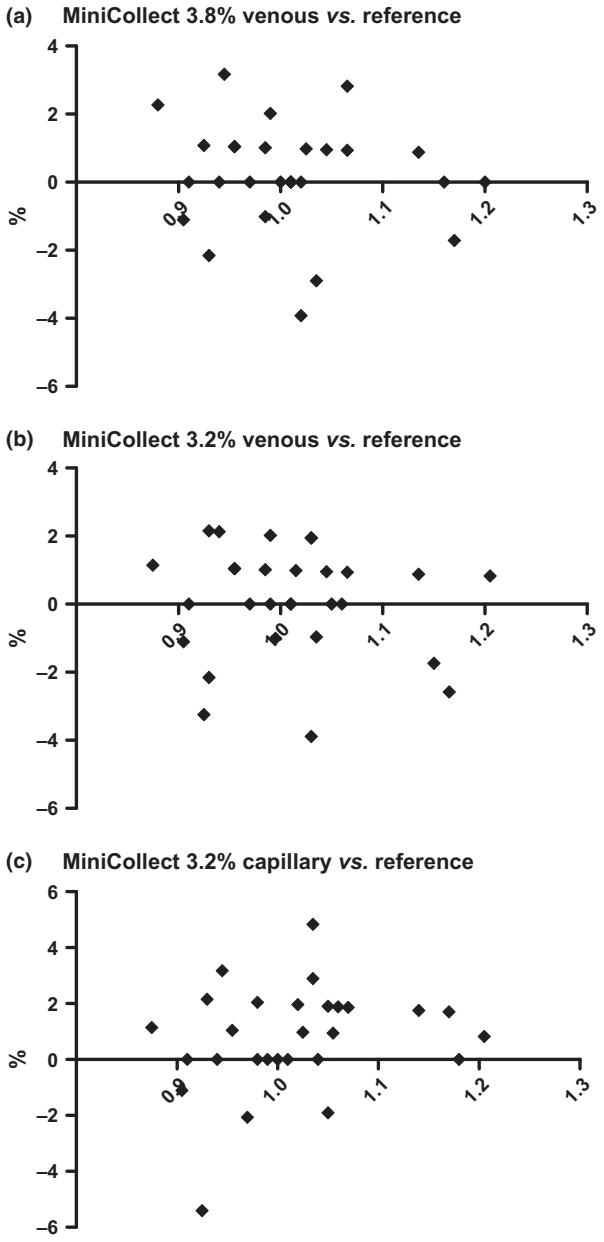

Figure 1. PT (INR) results obtained after using (a) small MiniCollect 3.8% tubes, (b) small MiniCollect 3.2% tubes, and (c) capillary sampling and small MiniCollect 3.2% tubes. The results are presented as percentage differences between investigated tubes and reference tubes *vs*. average (Bland–Altman plot).

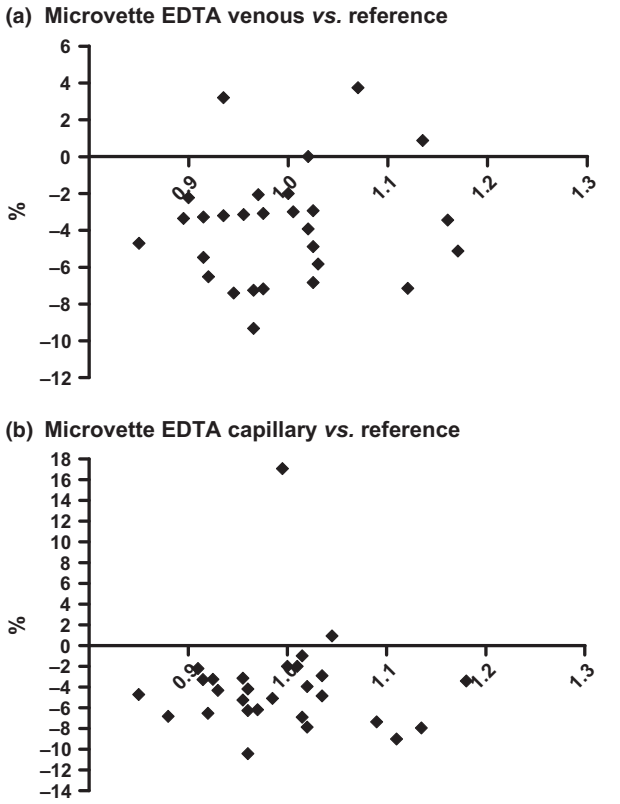

Figure 2. PT (INR) results obtained with (a) venous and (b) capillary samples in small Microvette EDTA tubes. The results are presented as percentage differences between investigated tubes and reference tubes *vs*. average (Bland–Altman plots).

whether or not these tubes are suitable for coagulation assays [17–19]. Horsti [18, 19] showed that EDTA tubes might be used with Owren's method instead of citrate tubes for monitoring PT (INR) in patients receiving oral anticoagulants. He concluded that the main reason for the absence of a problem with EDTA is the small sample volume required for Owren's method. However, in our study, samples in Microvette EDTA tubes, as well as the capillary samples in the MiniCollect tubes, were additionally diluted before the capillary method was run on the Sysmex CS-2100i. Nevertheless, the results appeared to be incorrect. However, this did not seem to influence the results associated with the MiniCollect tubes to the same extent, and they were more consistent with the reference results ($P = 0.04$). The most common anticoagulant used in coagulation testing is citrate at a concentration of 3.2% or 3.8%. In Sweden, the organization for national quality assurance in laboratory medicine recommends that laboratories use 3.2% citrate for coagulation testing [20], as also recommended

in Clinical and Laboratory Standards Institute (CLSI) guidelines [21]. However, samples in tubes with 3.8% citrate may still appear in the laboratory, and therefore, we included tubes with 3.8% citrate for comparison.

The reference range for PT (INR) in our laboratory is <1.2. As all 29 volunteers were healthy, only one subject had PT (INR) 1.2 (which may be considered outside the reference range). The results in other tubes varied between 1.14 and 1.21 (with the lowest values obtained from the two EDTA tubes). In spite of such findings, it is not possible to conclude from this example whether the use of EDTA microtubes would lead to potential misclassification of PT (INR) as normal.

The results of this study suggest that tubes intended for capillary samples might also be used for venous samples, as the PT (INR) values from venous samples in capillary tubes showed better agreement with the reference PT (INR) value than the PT (INR) values from capillary samples. This would open up the possibility for both small-volume sampling and sampling from places other than the arm fold, such as the tip of the finger, which may be desirable for some patients undergoing regular PT (INR) testing.

The main limitation of the study is its size, as a limited number of healthy volunteers were included. It would be beneficial to carry out the study with samples from warfarin-treated patients and with PT (INR) in the therapeutic range. This may be particularly important considering that Fiebig *et al.* [2] reported a bias of up to 10% at higher INR values when comparing plastic *vs.* glass tubes.

Nevertheless, it seems from our results that venous samples in 3.2% citrated MiniCollect tubes may be an appropriate solution if small-volume sampling is preferred. On the other hand, capillary samples in the same tubes resulted in a higher level of uncertainty as regards PT (INR) and therefore should be used only exceptionally, when other ways of sampling are not possible. Microvette EDTA tubes should not be used in connection with PT (INR) testing.

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CONFLICT OF INTERESTS

J. P. Antovic has received lecture honoraria from Stago, Siemens and Roche. None of the other authors declare any conflict of interests.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Results for individual samples as well as *P*-values from the two-sided paired Students *t*-test.