The Effect of 3.2% and 3.8% Sodium Citrate on Specialized Coagulation Tests

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● Context.—Coagulation testing is challenging and depends on preanalytic factors, including the citrate buffer concentration used.

Objective.—To better estimate preanalytic effects of the citrate buffer concentration in use, the difference between results obtained by samples with 3.2% and 3.8% citrate was evaluated.

Design.—In a prospective observational study with 76 volunteers, differences related to the citrate concentration were evaluated. For both buffer concentrations, reference range intervals were established according to the recommendations of the C28-A3 guideline published by the Clinical and Laboratory Standards Institute.

Results.—In our reagent-analyzer settings, most parameters evaluated presented good comparability between citrated samples taken with 3.2% and 3.8% trisodium buffer. The ellagic acid containing activated partial thromboplastin time reagent (aPTT-FS) indicated a systemic and proportional difference between both buffer concentrations, leading to an alteration in its reference ranges. Further, a confirmation test for lupus anticoagulant assessment (Staclot LA) showed only a moderate correlation ($r_p = 0.511$) with a proportional deviation between both citrate concentrations. Further, a statistically significant difference was found in the diluted Russell viper venom time confirmation testing, coagulation factors V and VIII, and the protein C activity, which was found to be of minor clinical relevance.

Conclusions.—With caution regarding the potential impact of the reagent-analyzer combination, our findings demonstrate the comparability of data assessed with 3.2% and 3.8% buffered citrated plasma. As an exception, the aPTT-FS and the Staclot LA assay were considerably affected by the citrate concentration used. Further studies are required to confirm our finding using different reagent-analyzer combinations.

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Despite a high degree of standardization, coagulation tests largely depend on preanalytic factors. Apart from artifacts acquired during blood drawing and transportation, the in vitro anticoagulant used has a potential impact on the coagulation test result. Usually, a 9:1 blood to sodium citrate ratio is used with a buffer range between 3.2% and 3.8%. The 3.2% buffered sodium citrate binds less assay-added calcium than 3.8% buffered sodium citrate, therefore clotting times tend to be shorter in 3.2% than 3.8% buffered sodium citrate. Although there are no exact data available, today 3.2% buffered citrate is rather widely used. A survey conducted in 2001 revealed that 156 of 593 US hospitals (26%) used the 3.8% buffered citrate for prothrombin time (PT) analysis. The 3.8% sodium citrated plasma appears to alter the International Sensitivity Index (ISI) of some thromboplastin times but is still the preferred material for special platelet function tests. For practical reasons, each institution usually uses 1 citrate concentration level for both coagulation testing and platelet function testing. Most recommendations are based on studies evaluating various PT and activated partial thromboplastin time (aPTT) reagents. The buffer concentration might amplify the effect of preanalytic factors, including the hematocrit or underfilling or overfilling of collecting tubes. Here, 3.2% buffered citrate tubes might be less influenced by underfilling than 3.8% buffered tubes during assessment of the global assays aPTT or PT.

However, precise knowledge about their impact on specialized coagulation tests is lacking. Overwhelming amounts of studies were conducted with these 2 buffer concentration ranges. To provide comparative data, we conducted this observational study for estimating the effects on a broad panel of coagulation tests as well as on their reference value ranges.

MATERIALS AND METHODS

Subjects

This prospective observational study was conducted at the Medical University of Vienna (Vienna, Austria) between September 2015 and July 2016. After we obtained written informed consent, 76 apparently healthy adult volunteers were included in the study. Volunteers under-
Table 1. Overview of Methods Used for Assessment

| Method                                      | Unit       | Analyzer                                      | Reagents                                         | Calibrators                                      |
|---------------------------------------------|------------|-----------------------------------------------|--------------------------------------------------|--------------------------------------------------|
| Prothrombin time                           | s          | STA-R Evolution                              | Thromborel S                                  | Standard Human Plasma                            |
| Fibrinogen                                  | mg/dL      | STA-R Evolution                              | STA-Liquid Fib                                 | NA                                               |
| aPTT-LA                                     | s          | STA-R Evolution                              | STA-aPTT                                       | NA                                               |
| aPTT-FS                                     | s          | STA-R Evolution                              | PTT-LA                                         | NA                                               |
| dRVVT                                       | s          | STA-R Evolution                              | Dade Actin FS aPTTb                            | NA                                               |
| dRVVTConfirm                                | s          | STA-R Evolution                              | Staclot DRVV Screen                           | NA                                               |
| aPTT-LAConfirm                              | s          | MC10 PLUS                                    | PTT-LA (with phospholipids)                      | Staclot LA                                        |
| Antithrombin III                            | %          | STA-R Evolution                              | Stachrom ATIII                                 | STA Unicalibrator                                |
| VWF-antigen                                 | %          | STA-R Evolution                              | Stastest VWF Ag                                 | VWF-Ag Calibrator                                |
| Factor II, factor V                         | %          | Sysmex CA-7000a                               | Thromborel Sl, deficient plasma                 | STA Unicalibrator                                |
| Factor VII                                  | %          | Sysmex CA-7000b                               | Thromborel Sl, deficient plasma                 | Standard Human Plasma                             |
| Factor X                                    | %          | Sysmex CA-7000b                               | Thromborel Sl, deficient plasma                 | STA Unicalibrator                                |
| Factor VIII/factor IX                       | %          | Sysmex CA-7000b                               | Dade Actin FS aPTTb, deficient plasma           | STA Unicalibrator                                |
| Factor XI                                    | %          | Sysmex CA-7000b                               | Dade Actin FS aPTTb, deficient plasma           | STA Unicalibrator                                |
| Factor XII                                   | %          | Sysmex CA-7000b                               | Dade Actin FS aPTTb, deficient plasma           | Standard Human Plasma                             |
| Protein C activity                          | %          | ACL-TOP                                      | Biophen Protein C5                             | STA Unicalibrator                                |
| Protein S activity                          | %          | STA-R Evolution                              | Staclot Protein S                               | STA Unicalibrator                                |
| Protein S antigen                           | %          | ACL-TOP                                      | Free Protein S                                  | Hemosil Calibration Plasma                       |

Abbreviations: aPTT-A, activated partial thromboplastin time; aPTT-FS, ellagic acid containing activated partial thromboplastin time reagent; aPTT-LA, lupus-sensitive activated partial thromboplastin time; aPTT-LAConfirm, lupus-sensitive activated partial thromboplastin time confirmation testing; dRVVT, diluted Russell viper venom time; dRVVTConfirm, diluted Russell viper venom time confirmation testing; NA, not applicable; VWF, von Willebrand factor antigen.

Methods

All analyses were executed under standardized conditions at the Department of Laboratory Medicine, which maintains a certified (according to ISO [International Organization for Standardization] 9001:2008) and accredited (according to ISO 15189:2008) quality management system. Using the same calibration settings, 3.2% and 3.8% buffered citrate samples were consecutively analyzed. Table I represents an overview of the analyzer platforms, reagents, calibrators, and controls used. The reagent-analyzer combinations were chosen with respect to measurement accuracy and validity, assays’ robustness and practicability during the parameters’ introduction phase at our laboratory. All assay procedures were performed according to the manufacturer’s recommendations. Automatic multidilution procedures were applied for factor testing. The aPTT-LA (lupus-sensitive reagent) for lupus anticoagulant (LAC) confirmation testing was analyzed by using plasma with and without a reagent containing phosphatidylethanolamine. Lupus anticoagulant confirmation testing was considered positive when the difference between both aPTT-LA clotting times was greater than 8 seconds (aPTT-LADifference, Staclot LA [Diagnostica Stago S.A.S, Asnières sur Seine, France]) or with the ratio between the neat diluted Russell viper venom time (dRVVT) and the dRVVTConfirm (dRVVTRatio, Staclot DRVV Screen/Staclot DRVV Confirm) was greater than 1.25.

Statistical Analysis

Numeric values are given as median with the interquartile range and are analyzed with the Wilcoxon rank sum test and the Spearman rank correlation test (r). The Passing-Bablok regression analysis was applied with bootstrapped confidence intervals. To ensure linearity between 2 tested parameters, a CuSum test was applied. Further, Bland-Altman plots are used to assess agreement between the 2 methods (3.2% and 3.8% citrate) and scatterplots are applied to assess the linearity between the 2 methods. To establish the reference interval, occurrence of Gaussian distribution (Shapiro-Wilk test) and the symmetry of skewness were assessed. Outliers were detected with the Tukey test. If necessary, a logarithm transformation or a Box-Cox transformation was performed. Reference intervals were calculated by using the robust method in accordance with the Clinical and Laboratory Standards Institute guideline C28-A3, which uses a bootstrapping approach. Statistical significance was defined as P values less than .05 (2-tailed). Where appropriate, the Bonferroni-Holm method was applied to adjust for an error related to multiple testing. Data were
Abbreviations: aPTT-A, activated partial thromboplastin time; aPTT-FS, ellagic acid containing activated partial thromboplastin time reagent; aPTT-LA, lupus sensitive activated partial thromboplastin time; aPTT-LADifference, lupus sensitive activated partial thromboplastin time confirmation testing difference; dRVVT, diluted Russell viper venom time; dRVVTConfirm, diluted Russell viper venom time confirmation testing; dRVVTRatio, diluted Russell viper venom time confirmation testing ratio.

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Coagulation parameters tested showed good comparability between both citrate buffer concentrations. The aPTT-FS presented with a systematic and proportional deviation and therefore the citrate buffer concentration had a significant impact on this measurement. Further, the citrate buffer concentration significantly affected the upper reference range limit of the aPTT-FS. In comparison to the aPTT-A (activated partial thromboplastin time), a pronounced effect of the buffer concentration on the aPTT-FS was also found by Adcock et al.6

Figure 1. Bland-Altman plots of selected parameters using 3.2% and 3.8% citrate. a, aPTT-FS (ellagic acid containing activated partial thromboplastin time reagent). b, dRVVT\textsubscript{Confirm} (diluted Russell viper venom time confirmation testing). c, dRVVT\textsubscript{Ratio} (ratio between the neat dRVVT and the dRVVT\textsubscript{Confirm}). d, Factor V. e, Factor VIII. f, Protein C activity.
One might speculate that calculated coagulation parameters are more sensitive to alterations of the citrate buffer concentration, since 2 or more factors are used, each having a potential error itself. The aPTT-LADifference presented only a moderate correlation, with a systemic difference between both citrate concentrations. Therefore, we advise special caution when comparing data of aPTT-LA confirmation testing. Interestingly, this effect was not seen in dRVVT confirmation testing. However, the dRVVTConfirm clotting time was considerably shortened in the higher citrate buffer concentration, contrary to expectations. In none of the cases tested was a false-positive result detected in either dRVVT Ratio or aPTT-LADifference confirmatory testing. Furthermore, a statistically significant difference was found in dRVVTConfirm, dRVVT Ratio, factor V, factor VIII, and protein C activity, but their absolute difference was in a minor range and no deviation could be detected in regression analysis.

In the literature, there are several publications reporting a significant influence of the citrate concentration on the aPTT or PT, but also publications that do not find any alteration in regard to the citrate concentration used. Apart from the parameter of inherent susceptibility to alterations of the citrate concentration, this might indicate that different reagent-analyzer combinations have different sensibilities in regard to the citrate concentrations used. For 60 patients receiving oral anticoagulants, the relative difference of the PT between both buffer concentrations ranged from 3.7% to 20% when measured under similar conditions using different reagent-analyzer combinations. Aside from the sodium concentration, the amount of magnesium or other electrolytes within the buffer might also have a significant influence on the comparability of parameters. In this evaluation, we assessed a broad spectrum of coagulation parameters by using a single specific reagent-analyzer combination for each parameter (Table 1). The reagent-analyzer combination itself might have an impact on the measurement, and therefore our results are not generalizable for other settings. Since we evaluated healthy volunteers without any coagulation disorders or anticoagulant therapy, we are not able to estimate the potential difference in pathologic samples. The data regarding the influence of the citrate buffer concentration on monitoring anticoagulation are nebulous. Adcock et al found in 5% of examined samples a significant deviation in INR (international normalized ratio) determination, due to different citrate buffer concentrations. Further, Payne et al did not observe any statistically significant influence of the citrate concentration on low-molecular-weight heparin monitoring in patients with pulmonary embolism or deep vein thrombosis.

In conclusion, the alteration of 3.2% and 3.8% buffered sodium citrate on specialized coagulation tests in our setting was of limited relevance. As an exception, the aPTT-FS and aPTT-LADifference were significantly affected by the citrate concentration used. Our findings demonstrate the comparability of data assessed with these citrate concentration ranges.

Figure 2. Scatterplot of the aPTT-FS using 3.2% and 3.8% citrate. Abbreviation: aPTT-FS, ellagic acid containing activated partial thromboplastin time reagent.

Figure 3. Graphical assessment of the aPTT-LADifference using 3.2% and 3.8% citrate. a, Bland-Altman plot. b, Scatterplot. Abbreviations: aPTT-LA, activated partial thromboplastin time lupus-sensitive reagent; aPTT-LADifference, difference between clotting times of the aPTT-LA with and without a phosphatidylethanolamine-containing reagent.
Table 4. Reference Range (Lower and Upper Limit) of Assessed Parametersa,b

| Parameter               | N  | 3.2% Citrate | 3.8% Citrate |
|-------------------------|----|--------------|--------------|
| Prothrombin time        | 76 | 12.1 (12.0–12.3) | 12.1 (11.9–12.2) |
| Fibrinogen              | 78 | 162 (146–178) | 164 (149–179) |
| Antithrombin III        | 77 | 89 (87–92) | 89 (87–91) |
| vWF-antigen             | 73 | 57 (54–62) | 56 (52–60) |
| aPTT-A                  | 78 | 176 (157–196) | 178 (158–201) |
| aPTT-FS                 | 77 | 39.4 (38.5–40.3) | 39.4 (38.5–40.2) |
| aPTT-LA                 | 79 | 32.8 (32.1–33.7) | 32.1 (31.2–33.0) |
| aPTT-LAConfirm          | 63 | 34.2 (33.1–35.5) | 33.8 (32.7–35.0) |
| fVIIa                    | 76 | 44.0 (43.0–44.9) | 44.3 (43.2–45.3) |
| dRVVT 70                | 62 | Not performed | Not performed |
| dRVVT 35.4              | 35.4 (34.3–36.6) | 35.6 (34.4–36.8) |
| dRVVT 35.6              | 35.7 (35.5–36.9) | 35.8 (35.6–36.9) |
| dRVVT 35.8              | 35.9 (35.7–36.9) | 36.0 (35.8–36.9) |
| dRVVT 36.0              | 36.0 (35.8–36.9) | 36.1 (35.9–36.9) |
| dRVVT 36.6              | 36.1 (36.0–36.9) | 36.2 (36.1–36.9) |
| Factor II               | 76 | 80 (75–85) | 79 (74–84) |
| Factor V                | 76 | 76 (69–83) | 75 (69–81) |
| Factor VII              | 76 | 58 (51–64) | 57 (50–63) |
| Factor X                | 76 | 73 (66–79) | 72 (65–75) |
| Factor VIII              | 76 | 71 (65–79) | 72 (65–79) |
| Factor IX               | 76 | 169 (158–181) | 163 (152–173) |
| Factor XI               | 76 | 65 (60–70) | 68 (64–73) |
| Factor XII              | 76 | 63 (55–73) | 66 (59–75) |
| Protein C activity      | 75 | 157 (150–166) | 154 (148–162) |
| Protein S activity      | 73 | 136 (129–143) | 137 (129–145) |
| Protein S antigen       | 76 | 63 (59–71) | 64 (59–71) |

Abbreviations: aPTT-A, activated partial thromboplastin time; aPTT-FS,ellanic acid containing activated partial thromboplastin time reagent; aPTT-LA, lupus-sensitive activated partial thromboplastin time; aPTT-LAConfirm, lupus-sensitive activated partial thromboplastin time confirmation testing; aPTT-LADifference, lupus-sensitive activated partial thromboplastin time confirmation testing difference; dRVVT, diluted Russell viper venom time; dRVVTConfirm, diluted Russell viper venom time confirmation testing; dRVVRTRatio, Diluted Russell viper venom time confirmation testing ratio; vWF, von Willebrand factor antigen.

a Lower limit/upper limit.
b Outliers were detected with the Tukey test.

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