

MULTICENTER EVALUATION OF A BILAYER POLYMER BLOOD COLLECTION TUBE FOR COAGULATION TESTING : EFFECT ON ROUTINE HEMOSTASIS TEST RESULTS AND ON PLASMA LEVELS OF COAGULATION ACTIVATION MARKERS

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INTRODUCTION

- For years, laboratories have used evacuated glass tubes for blood collection. To improve the safety of blood collection and to avoid inherent hazards associated with glass, such as injuries due to broken tubes or tubes broken during centrifugation, plastic tubes have been developed for laboratory medicine. The increasing demand for plastic tubes also has an ecological basis since the amount of waste after incineration is lower than in the case of glass tubes.
- However, change of blood collection tubes for coagulation testing raises concerns because of the potential for "in vitro" activation of the coagulation leading to alteration of clotting times and single factor levels due to consumption. The same apply for interactions with antithrombotic drugs, as reported with the first generation of such tubes. In addition, the well-known permeability of plastics to liquids and/or gas can induce modifications not only of the blood to citrate anticoagulant solution ratio (initially 9 vol/1 vol) but also of the citrate concentration (initially 0.109M or 0.129M) and could produce a drop in the pH.

STUDY AIM

- The aim of this multicenter study was to compare the results of different hemostasis tests, ie prothrombin time (PT) / INR, activated partial thromboplastin time (APTT), factor V and anti-FXa activity, evaluated in an evacuated bilayer polymer (polyethylene terephthalate outer layer and polypropylene inner layer) tubes [Vacuette®, Greiner Bio-One, Kremsmünster, Austria] with those measured in evacuated siliconized glass tubes [Vacutainer®, Becton - Dickinson, Le Pont-de-Claix, France] containing the same citrate concentrations (0.109M and 0.129M)^[1].

PATIENTS

- For that purpose, blood was collected in five centers from untreated patients (n = 60) and from patients on traditional anticoagulant treatment, ie oral anticoagulant (n = 168), unfractionated heparin (n = 111) or a low molecular weight derivative (n = 108). The sampling order of the different tubes was randomly defined.

METHODS

- The sampling procedure was performed in accordance with the guidelines of the GEHT^[2]. Tubes were immediately centrifuged twice at 3000 × g and +12°C for 15 minutes. Routine coagulation tests were immediately performed, and the remaining plasma was frozen in aliquots at -80°C. Frozen aliquots were then shipped in dry ice to a single center for the measurement of molecular markers of coagulation activation. Routine coagulation tests, ie prothrombin time (PT), activated partial thromboplastin time (APTT), factor V (FV) and anti-factor Xa activity were evaluated in each participating laboratory using his own routine techniques (reagents and instruments). The plasma levels of thrombin-antithrombin complexes (TAT) and prothrombin fragment 1 + 2 (F1+2) were evaluated in a single center using Enzygnost TAT and Enzygnost F1+2 reagents respectively [both from Dade Behring, Paris-la Défense, France].
- The stability of the samples was evaluated for the routine tests over a 6-hour period, by measuring PT, APTT, and factor V immediately after centrifugation, 3 hours and 6 hours after the first measurement.
- The test results were expressed as the mean values with SDs (except for TAT as the median values with ranges). Comparison of test results obtained in polymer and glass tubes was performed using the paired Student's T-test or the Mann-Whitney's U-test when appropriate. The ANOVA was used to evaluate any time-dependent evolution of the results. A pvalue smaller than 0.05 was considered to be statistically significant. In addition, data were compared according to Bland-Altman to assess the clinical significance of the differences^[3].

RESULTS

- Test results obtained in plastic tubes were not significantly different from those in glass tubes, except for INR when a high ISI thromboplastin was used (p < 0.0001 in the case of tubes containing 0.129 M sodium citrate) and for APTT (p < 0.05 for both citrate concentrations) in untreated patients (Table 1) and patients on anticoagulant therapy (Table 2). However, these differences had no clinical relevance (Bland - Altman analysis). In addition, no effect of aging of the polymer tubes on the test results could be demonstrated in treated patients, as suggested by the lack of significant discrepancy in the test results obtained in polymer tubes at beginning or end of their shelf life (Table 2).
- Significant changes in test results were demonstrated for PT, APTT and factor V over a 6-hour period that was of the same order of magnitude for all tubes (not shown).
- The plasma levels of F1+2 and TAT complexes, measured in a subset of 30 untreated patients, were significantly lower when blood was collected in polymer than in glass tubes, for both citrate concentrations (Table 3).

TABLE 1. Prothrombin time (PT), activated partial thromboplastin time (APTT), and coagulation factor V in 60 normal subjects. In the five participating centers, blood was collected from 12 subjects into evacuated polymer and glass tubes containing both 0.109M and 0.129M sodium citrate solution. All tubes were at beginning of their shelf life. Results were expressed as the mean with standard deviation.

TABLE 2. Hemostasis tests results in polymer and glass tubes obtained from patients on traditional anticoagulant therapy, ie international normalized ratio (INR) in patients on oral anticoagulant treatment, APTT and anti-FXa activity in patients on unfractionated heparin (UFH) and anti-FXa in patients on low molecular weight heparin (LMWH) sampled in the five participating centers. Two centers evaluated tubes containing 0.109M sodium citrate and three centers evaluated tubes containing 0.129M sodium citrate. The effect of aging of the polymer tube on the test result was investigated by using two lots of polymer tubes containing 0.109M sodium citrate at 11 months (beginning of shelf life = BSL) and at 1 month (end of shelf life = ESL) to expiry. The results were expressed as the mean values with standard deviation. Analytical comparison was made using the Student's t-test.

TABLE 3. Prothrombin fragment 1 + 2 (F1+2) and thrombin antithrombin complexes (TAT) measured in 30 untreated patient sampled in the five participating centers into evacuated polymer and glass tubes containing 0.109M and 0.129M sodium citrate. All tubes were at the beginning of their shelf life. Results were expressed as the mean with standard deviation for F1+2 and as the median with range for TAT since data were not normally distributed.

CITRATE CONCENTRATION	0.109 M		0.129 M		
	TUBE	POLYMER	GLASS	POLYMER	GLASS
PT	(RATIO)	1.02 ± 0.05	1.03 ± 0.05	1.02 ± 0.06	1.02 ± 0.06
	(%)	95.7 ± 9.7	95.8 ± 9.5	97.6 ± 10.9	98.10 ± 11.20
APTT	(RATIO)	0.99 ± 0.10*	1.00 ± 0.10	1.00 ± 0.11**	1.01 ± 0.10
	(seconds)	30.6 ± 3.3 *	30.9 ± 3.4	30.8 ± 3.4 **	31.30 ± 3.40
Factor V (%)		100.7 ± 18.8	101.8 ± 18.4	99.7 ± 17.5	99.30 ± 18.80

* : p < 0.001, ** : p < 0.0001 (Polymer versus Glass)

CITRATE CONCENTRATION	0.109 M				0.129 M			
	TUBE	n	BSL POLYMER	ESL POLYMER	GLASS	n	BSL POLYMER	GLASS
INR		57	2.20 ± 0.77	2.18 ± 0.75	2.16 ± 0.75	111	2.34 ± 0.87 **	2.31 ± 0.85
APTT (RATIO) UFH		57	2.14 ± 0.70 *	2.14 ± 0.69 *	2.20 ± 0.72	54	2.18 ± 0.84 *	2.23 ± 0.85
ANTI - FX a (IU / mL) UFH		57	0.41 ± 0.28	0.40 ± 0.26	0.40 ± 0.26	54	0.43 ± 0.23	0.43 ± 0.23
ANTI - FX a (IU / mL) LMWH		50	0.42 ± 0.35	0.40 ± 0.33	0.40 ± 0.34	58	0.56 ± 0.40	0.55 ± 0.40

* : p < 0.05, ** : p < 0.01 (Polymer versus Glass)

CITRATE CONCENTRATION	0.109 M		0.129 M		
	TUBE	POLYMER	GLASS	POLYMER	GLASS
F 1+2 (nmol / L)		1.11 ± 0.26 **	1.15 ± 0.27	1.07 ± 0.24 *	1.1 ± 0.25
TAT (ng / mL)		1.59 [0.10 - 7.58]***	2.35 [0.10 - 5.58]	1.41 [0.1 - 4.64]**	1.81 [0.10 - 5.61]

* : p < 0.05, ** : p < 0.01, *** : p < 0.005 (Polymer versus Glass)

REFERENCES

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CONCLUSIONS

These results suggest that samples collected into the Vacuette® polymer tubes allow accurate routine hemostasis testing both in untreated patients and in patients on traditional anticoagulant treatment during the whole shelf life indicated by the manufacturer.

In addition, the coagulation system appeared to be less activated in polymer tubes than in glass tubes containing similar citrate concentration, even if the markers remained within the expected range in all cases.