A Comparison of Glass and Plastic Blood Collection Tubes for Routine and Specialized Coagulation Assays

A Comprehensive Study

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• ***Context.*—Blood collection tubes made from plastic are beginning to replace glass tubes. Coagulation test results can be influenced easily by preanalytic factors, including exposure to surfaces that activate the clotting cascade.**

***Objective.*—To compare the effects of the blood collection tube material on 22 coagulation assays performed in clinical laboratories.**

***Design.*—Paired blood samples from 28 healthy volunteers were drawn into BD Vacutainer Glass Citrate Tubes and BD Vacutainer Plus Plastic Citrate Tubes, and the results of coagulation assays were determined in parallel.**

***Results.*—No statistically significant differences were observed between glass and plastic for 14 assays: prothrombin time (and international normalized ratio); activated partial thromboplastin time; activated protein C resistance; antithrombin activity; factors II, V, VIII, and IX; α_2-antiplasmin; plasminogen activity; von Willebrand factor antigen; ristocetin cofactor; thrombin time; and reptilase time. Statistically significant differences were found for fibrinogen; chromogenic protein C activity; protein S activity; PTT-LA lupus anticoagulant–sensitive activated partial thromboplastin time; and factors VII, X, XI, and XII. Mean differences ranged from 0.4% to 5.5% and were unlikely to be of clinical significance.**

***Conclusions.*—The results of this study suggest that plastic tubes can be used in place of glass tubes for a wide variety of coagulation assays.**

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Like all laboratory tests, coagulation assays can be affected by a large variety of preanalytic variables, including the material used to manufacture the blood collection tube. Historically, tubes made of glass have been used to collect blood samples. Since the coagulation cascade can be activated by contact of blood with glass surfaces, these tubes are siliconized to prevent glass-induced coagulation activation.[1] Recently, blood collection tubes made of a variety of plastic materials have started to replace glass tubes in many laboratories. Plastic tubes have increased shock resistance and tolerance of higher centrifugation speeds than glass tubes, providing improved safety for laboratory employees. The reduced solid waste after incineration of plastic tubes addresses environmental concerns,[2] and the slight flexibility of plastic tubes makes them better suited for use in an automated laboratory with robotics-based sample handling.[3,4]

In spite of the increasing use of plastic tubes for coagulation testing, and in spite of the well-known ability of certain tube materials to activate the coagulation cascade more than other materials, there are only a limited number of independent reports in the peer-reviewed literature comparing the effects of plastic versus glass collection tubes on routine and esoteric coagulation tests. We therefore performed a comprehensive study of the effects of glass versus plastic blood collection tubes on the results of 22 coagulation assays. Our study encompassed routine, high-volume tests performed in many laboratories offering standard testing menus, as well as more esoteric tests generally limited to specialized coagulation laboratories.

MATERIALS AND METHODS

Blood-Draw Tubes

Blood samples were drawn into either 4.5-mL BD Vacutainer Glass Citrate Tubes or 2.7-mL BD Vacutainer Plus Plastic Citrate Tubes (BD, Franklin Lakes, NJ). Both tubes contain 3.2% citrate and have a vacuum designed to collect blood in a 9:1 ratio of blood to citrate (4.5-mL plastic tubes are not available from this manufacturer, necessitating the use of 2.7-mL tubes). BD Vacutainer Plus plastic tubes consist of an outer layer made of polyethylene terephthalate (PET) plastic, which is shatter-resistant, and an inner layer made of polypropylene plastic. The inner polypropylene plastic layer contains the citrate and, when collected, the blood specimen. The dual-tube construction produces a more shatter-resistant tube with a smaller dead space.

Blood Samples

After informed consent was obtained, paired blood samples from 28 healthy blood donors were collected by trained technologists into 3 BD Vacutainer glass and 5 plastic tubes. Both samples were obtained from a single venipuncture, and the order of the tubes (plastic or glass) was alternated randomly. Paired samples were processed and analyzed at the same time. Specimens for routine coagulation testing (prothrombin time [PT], activated

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Table 1. Coagulation Assays, Instruments, and Reagents Used*

Test	Instrument	Reagent	Method Comment
PT	MDA-180 (bioMerieux)	Simplastin L (bioMerieux)	Optical clot detection
aPTT	MDA-180	Platelin L (bioMerieux)	Optical clot detection
Fibrinogen	MDA-180	Fibriquik (bioMerieux)	Clauss method
Activated protein C resistance	STA-R (Diagnostica Stago)	Coatest APC Resistance V (Chromogenix/DiaPharma)	aPTT-based with factor V–deficient plasma
Antithrombin activity	STA-R	Stachrom ATIII (Diagnostica Stago)	Thrombin-based chromogenic
Protein C chromogenic acivity	STA-R	Coamatic Protein C (Chromogenix/DiaPharma)	Chromogenic
Protein S activity	STA-R	Staclot Protein S (Diagnostica Stago)	aPTT-based functional assay
Lupus anticoagulant	MDA-180 (bioMerieux)	PTT-LA (Diagnostica Stago)	Dilute phospholipid aPTT
Factor II	MDA-180	Factor II–deficient plasma (Trinity Biotech)†	PT-based
Factor V	MDA-180	Factor V–deficient plasma (Precision Biologic)†	PT-based
Factor VII	MDA-180	Factor VII–deficient plasma (Precision Biologic)†	PT-based
Factor VIII	MDA-180	Factor VIII–deficient plasma (Precision Biologic)‡	aPTT-based
Factor IX	MDA-180	Factor IX–deficient plasma (Precision Biologic)‡	aPTT-based
Factor X	MDA-180	Factor X–deficient plasma (Precision Biologic)†	PT-based
Factor XI	MDA-180	Factor XI-deficient plasma (Trinity Biotech)‡	aPTT-based
Factor XII	MDA-180	Factor XII–deficient plasma (Trinity Biotech)‡	aPTT-based
Antiplasmin activity	MDA-180	α_2-Antiplasmin (bioMerieux)	Chromogenic
Plasminogen activity	MDA-180	Plasminogen (bioMerieux)	Chromogenic
von Willebrand factor antigen	MDA-180	Liatest vWF (Diagnostica Stago)	Latex immunoturbidimetric
Ristocetin cofactor	PACKS-4 (Helena Laboratories)	Ristocetin cofactor (ABP), lyophilized platelets (Helena Laboratories)	Platelet aggregometry
Thrombin time	ST4 (Diagnostica Stago)	Thromboquik (bioMerieux)	1.5 U/mL final concentration bovine thrombin
Reptilase time	ST4	Atroxin (Sigma Diagnostics)	5 U/mL final concentration Atroxin

* PT indicates prothrombin time; aPTT, activated partial thromboplastin time. Instrument and reagent manufacturers: bioMerieux, Durham, NC; Diagnostica Stago, Asnieres, France; Helena Laboratories, Beaumont, Tex; Chromogenix/DiaPharma, West Chester, Ohio; Trinity Biotech/Sigma Diagnostics, St Louis, MO; Precision Biologic, Dartmouth, Nova Scotia; ABP, Marlton, NJ.

† Prothrombin time factor assays also used Simplastin L PT reagent.

‡ Activated partial thromboplastin time factor assays also used Platelin L aPTT reagent.

partial thromboplastin time [aPTT], and fibrinogen) were processed within less than 2 hours of collection. All other samples were processed within less than 1 hour of collection. Centrifugation was performed at 1500*g* for 10 minutes. Assays for activated protein C resistance, chromogenic protein C, protein S, antithrombin, and some of the PT, aPTT, and fibrinogen pairs were performed immediately after processing. For the remainder of the assays, plasma was stored at −70°C until assayed. Only donors with no history of coagulation disorders and who reported no current anticoagulant therapy were allowed to donate.

Coagulation Assays

Table 1 lists the coagulation assays performed and the analyzers and reagents used to perform the tests. Assays were performed in accordance with the manufacturers' instructions by laboratory technologists following the laboratory's standard operating procedure. Quality controls were run every 4 hours for tests performed on the MDA-180 (bioMerieux, Durham, NC) and every 8 hours for the remaining tests.

Statistical Analysis

Statistical analysis was performed using Microsoft Excel software (Microsoft, Redmond, Wash), including 2-tailed paired *t* tests, Pearson correlation coefficients (*r*), and Bland-Altman plots.

RESULTS

Twenty-two different coagulation assays were performed on 28 paired samples drawn into glass and plastic tubes (Table 2). Paired *t* tests showed no statistically significant differences between glass and plastic for 14 assays: PT (and the international normalized ratio); aPTT; activated protein C resistance; antithrombin activity; factors II, V, VIII, and IX; α_2-antiplasmin; plasminogen activity; von Willebrand factor antigen; ristocetin cofactor; thrombin time; and reptilase time. Statistically significant differences ($P < .05$) were found for fibrinogen; chromogenic protein C activity; protein S activity; lupus anticoagulant–sensitive aPTT (PTT-LA); and factors VII, X, XI, and XII. The mean differences ranged from 0.4% to 5.5%; variations of this magnitude are unlikely to be of clinical significance. Pearson correlation coefficients (*r*) for all tests with $P < .05$ showed good correlation between results in plastic and glass: fibrinogen, $r = 0.99$; chromogenic protein C activity, $r = 0.98$; protein S activity, $r = 0.98$; PTT-LA, $r = 0.89$; factor VII, $r = 0.88$; factor X, $r = 0.96$; factor XI, $r = 0.92$; and factor XII, $r = 0.93$. Figures 1 through 4 show Bland-Altman bias plots for each of the 22 assays.

Two individuals had abnormally low results for acti-

Table 2. Coagulation Test Results for Specimens Collected into Glass and Plastic Blood Collection Tubes*			
	Glass	Plastic	*P* Value
PT, s	12.4 (0.35)	12.5 (0.32)	.33
INR	1.0	1.0	.34
aPTT, s	25.1 (2.39)	24.9 (2.36)	.10
Fibrinogen, mg/dL	336 (82)	345 (84)	<.001
Activated protein C resistance	2.38 (0.19)	2.39 (0.20)	.90
Antithrombin activity, %	115.2 (13.8)	115.9 (14.4)	.66
Protein C chromogenic activity, %	108.7 (16.4)	110.8 (16.9)	.002
Protein S activity, %	99.8 (30.2)	94.3 (27.3)	<.001
PTT-LA lupus anticoagulant, s	34.4 (3.43)	32.8 (3.10)	<.001
Factor II, %	117.1 (14.2)	114.9 (13.1)	.17
Factor V, %	101.1 (20.6)	104.6 (24.4)	.09
Factor VII, %	120.3 (29.7)	114.4 (29.7)	.04
Factor VIII, %	105.0 (26.2)	107.5 (24.5)	.47
Factor IX, %	109.0 (19.4)	110.1 (19.1)	.60
Factor X, %	123.3 (23.3)	126.5 (24.9)	.03
Factor XI, %	111.5 (18.4)	115.6 (18.2)	.008
Factor XII, %	96.1 (24.5)	100.2 (25.8)	.03
Antiplasmin activity, %	128.1 (8.3)	130.1 (7.9)	.20
Plasminogen activity, %	124.0 (15.0)	128.3 (11.8)	.08
von Willebrand factor antigen, %	121.3 (39.1)	125.6 (39.6)	.07
Ristocetin cofactor, %	125.6 (49.1)	124.3 (48.5)	.64
Thrombin time, s	18.63 (1.5)	18.65 (1.2)	.94
Reptilase time, s	18.3 (2.9)	18.7 (4.4)	.37

* Data are given as mean (SD); *P* values are for paired 2-tailed *t* tests. PT indicates prothrombin time; INR, international normalized ratio; and aPTT, activated partial thromboplastin time.

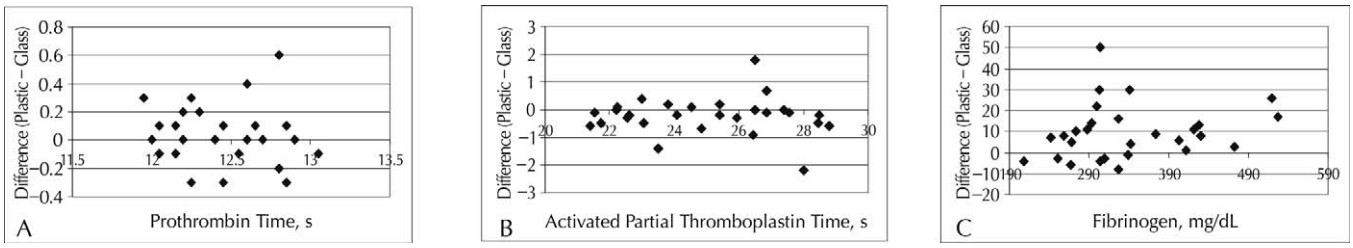

Figure 1. *Bland-Altman plots for routine coagulation assays (prothrombin time, activated partial thromboplastin time, and fibrinogen) for paired samples drawn into glass and plastic tubes. Values on the x-axis indicate the average of the result in plastic and the result in glass.*

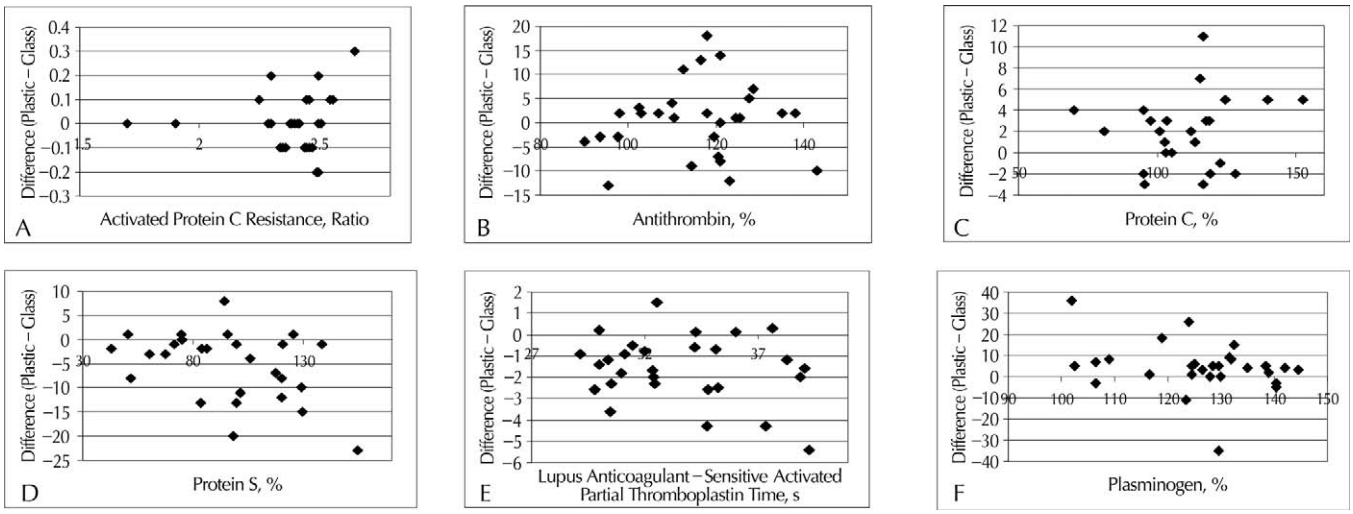

Figure 2. *Bland-Altman plots for hypercoagulation assays for paired samples drawn into glass and plastic tubes. Values on x-axis indicate the average of the result in plastic and the result in glass.*

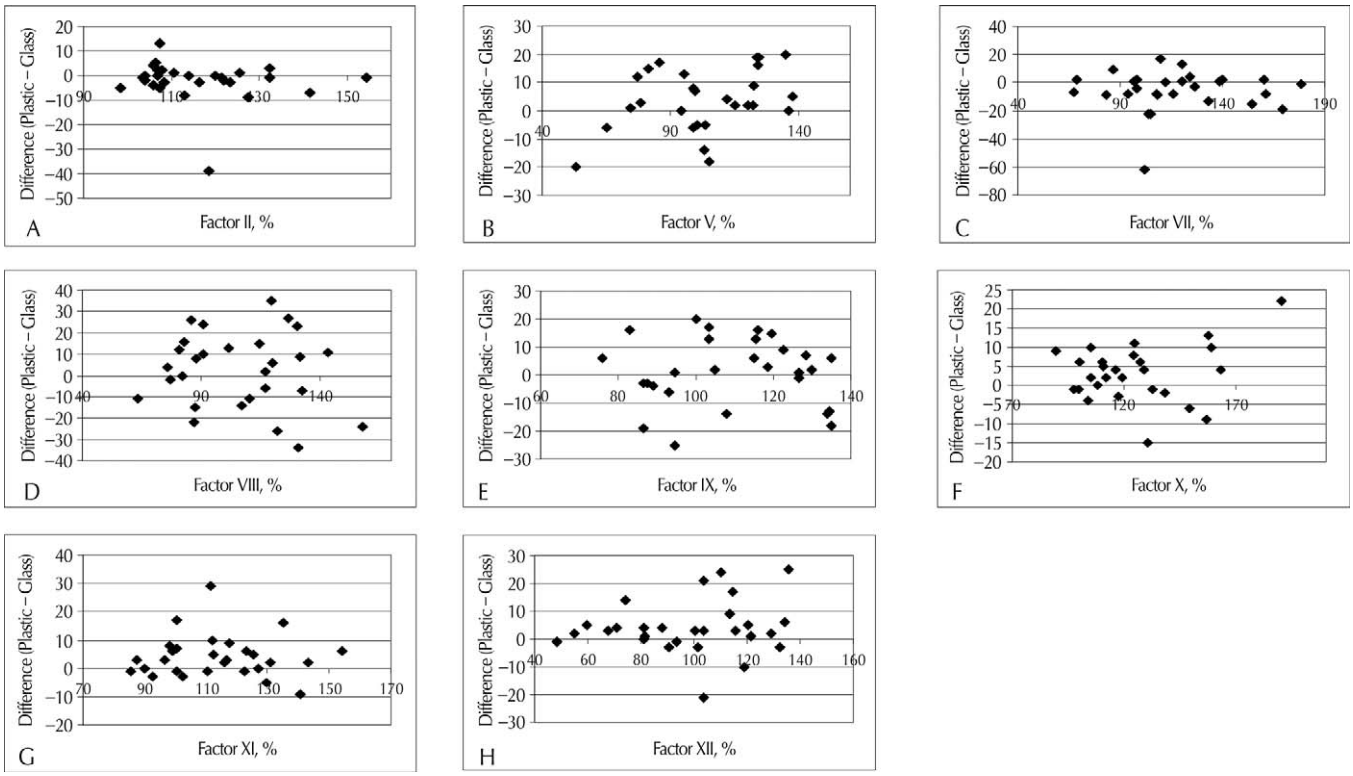

Figure 3. *Bland-Altman plots for coagulation factor assays for paired samples drawn into glass and plastic tubes. Values on x-axis indicate the average of the result in plastic and the result in glass.*

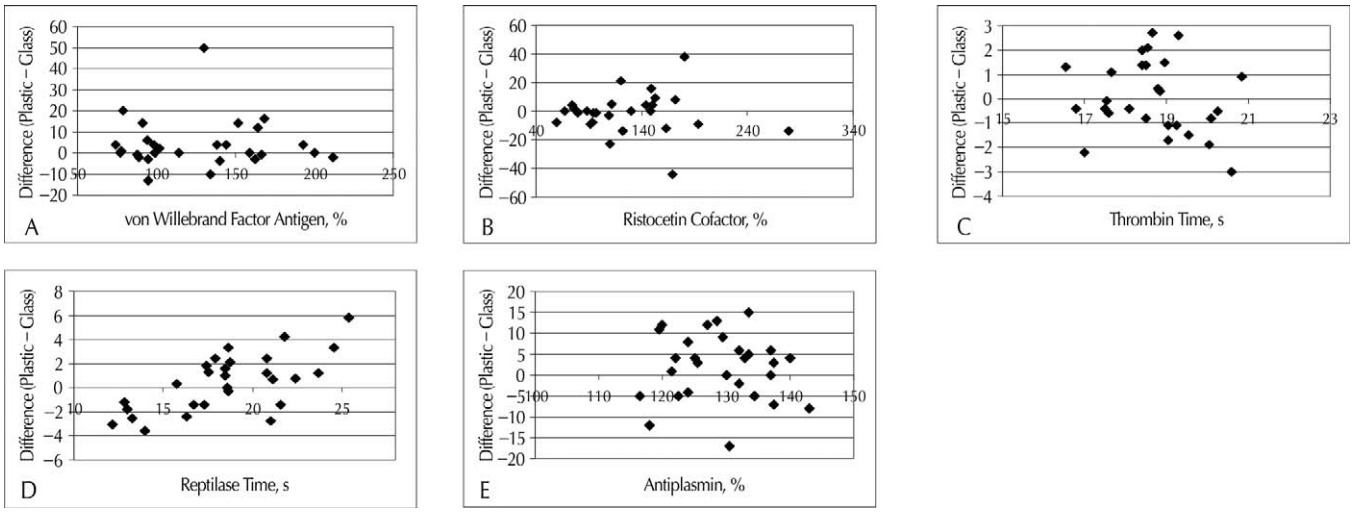

Figure 4. *Bland-Altman plots for coagulation assays used to diagnose bleeding diatheses for paired samples drawn into glass and plastic tubes. Values on x-axis indicate the average of the result in plastic and the result in glass.*

vated protein C resistance, the screening test for factor V Leiden. For both donors, results were identical in glass and plastic collection tubes. Five of the study participants were women on oral estrogen therapy, and as expected, their protein S values were decreased. The values in these samples with low protein S were very similar in the glass and plastic tubes (mean in glass, 56.2% [SD 8.8]; mean in plastic, 53.2% [SD 8.4]; paired 2-tailed *t* test, $P = .052$).

Table 3 shows the results of the present study compared with results of 2 other studies that investigated the effect of glass compared to plastic blood collection tubes on coagulation assays.

COMMENT

We have compared the effects of glass versus plastic blood collection tubes on the results of 22 coagulation assays and found no clinically significant differences. Overall, our findings are consistent with the reports of other investigators who generally also described no clinically significant differences in coagulation results obtained on

Table 3. Comparison of the Findings of This Study With Findings of 2 Other Reports*

	Present Study	Gosselin,[10] 2004†	Flanders,[9] 2003
PT, s	↔	↓	Not done
INR	↔	Not done	Not done
aPTT, s	↔	↓	Not done
Fibrinogen, mg/dL	↑	↓	↔
Activated protein C resistance	↔	Not done	↔
Antithrombin, %	↔	↓	↔
Protein C activity, %‡	↑	↓	↔
Protein S activity, %	↓	↑	↑
Lupus anticoagulant, s§	↓	↑	Not done
Factor II, %	↔	Not done	↔
Factor V, %	↔	Not done	↔
Factor VII, %	↓	Not done	↓
Factor VIII, %	↔	Not done	↓
Factor IX, %	↔	Not done	↔
Factor X, %	↑	Not done	↓
Factor XI, %	↑	Not done	↔
Factor XII, %	↑	Not done	↓
Antiplasmin activity, %	↔	Not done	Not done
Plasminogen activity, %	↔	↔	Not done
von Willebrand factor antigen, %	↔	Not done	↔
Ristocetin cofactor, %	↔	↔	↔
Thrombin time, s	↔	Not done	↑↑
Reptilase time, s	↔	Not done	Not done

* PT indicates prothrombin time; INR, international normalized ratio; aPTT, activated partial thromboplastin time; ↔, no statistically signficant difference between plastic and glass ($P > .05$); ↑, results in plastic tubes statistically significantly higher than in glass tubes ($P < .05$), but difference was, in the authors' opinion, unlikely to be of clinical significance; ↓, results in plastic tubes statistically significantly lower than in glass tubes ($P < .05$), but difference was, in the authors' opinion, unlikely to be of clinical significance; and ↑↑, results in plastic tubes statistically and (in the authors' opinion) clinically significantly higher than glass.

† Results are for BD plastic Vacutainer tubes; in addition, no clinically significant differences were reported for 3.2% sodium citrate VACUETTE sandwich tubes (Greiner Bio-one, Monroe, NC).

‡ Present study used chromogenic method; the other 2 studies used clotting method.

§ Present study used lupus anticoagulant-sensitive aPTT; the study by Gosselin and colleagues used dilute Russell viper venom time.

samples drawn into plastic tubes compared to glass tubes. Many of these studies were limited to routine, high-volume coagulation assays or to small numbers of more esoteric coagulation assays. D'Angelo and Villa[5] compared PT results in samples drawn into glass and PET plastic tubes (as opposed to the polypropylene plastic used for the inner lining of the tubes investigated in our study); they found statistically and clinically (differing by >10%) significantly lower values in glass tubes. Tripodi and colleagues[6] compared the PT and international normalized ratio obtained from specimens drawn into glass and plastic BD tubes, and found a statistically significant, but not clinically relevant difference between the 2 tube materials. Other groups have concentrated on subgroups of coagulation assays. Leroy-Matheron and colleagues[7] found no difference between glass and PET plastic collection tubes with regard to several markers of coagulation activation (prothrombin fragment 1.2, thrombin-antithrombin complexes, or D-dimers). In another study using PET plastic with up to 30 specimens, no significant difference was observed for anti-factor Xa, but prothrombin fragment 1.2 was higher and factor XIIa was lower in glass than in plastic, reaching statistical significance for some of the groups studied.[8]

To the best of our knowledge, only 2 other studies have investigated a similarly broad range of coagulation assays: Flanders and colleagues[9] compared the results of 19 esoteric coagulation assays in glass versus plastic Vacutainers using samples from 20 normal donors, and Gosselin and coworkers[10] determined the effects of tube material on the results of 15 coagulation assays in randomly selected patients. Table 3 compares our results with the findings of these 2 groups. The only difference between results obtained in glass and in plastic tubes that was both statistically and clinically (in the authors' opinion) significant was a prolongation of the mean thrombin time in plastic tubes in the study of Flanders et al.[9] In contrast, in our study, the thrombin time was prolonged by only 0.02 seconds in plastic compared to glass, a statistically (and clinically) insignificant difference. It can be speculated that the differing results could be attributed to the fact that the glass tube was always drawn before the plastic tube in the study by Flanders et al, whereas in our study the order of draw was randomized. It is also possible that the use of different reagents and instruments in the 2 studies accounts for the different findings.

Two types of plastic are commonly used to manufacture plastic blood collection tubes, polypropylene and PET. According to the manufacturer of the tubes used in the present study, PET is virtually unbreakable and is capable of maintaining a vacuum. On the other hand, polypropylene maintains a better liquid barrier than PET, thereby retaining the liquid citrate and maintaining the appropriate citrate concentration in the specimen. Liquid within PET tubes tends to evaporate.

Our present results comparing blood-draw tubes containing citrate as an anticoagulant are similar to findings in our previous reports comparing glass K_3EDTA and plastic K_2EDTA blood-draw tubes, in which we also found statistically significant differences between samples collected into glass versus plastic tubes for complete blood count, white blood cell differential, reticulocyte, and platelet activation parameters; however, the differences were unlikely to be of clinical significance.[4,11]

To our knowledge, this study is the first to compare the effects of glass versus plastic blood collection tubes on α_2-antiplasmin, reptilase time, PTT-LA assay, and chromogenic protein C activity. For the remaining analytes in this study, we believe this is the first report to compare glass and plastic using the reagent-instrument combination described herein, except that protein S, activated protein C resistance, and antithrombin results were reported previously.[9] Lastly, to our knowledge this is the first study to compare glass and plastic with the thrombin time since Flanders et al found a clinically significant difference for the thrombin time in this setting. A possible limitation of our study is that most test results were in the normal range. Further study involving more abnormal samples may be informative. Among the abnormal results in this study, we found no differences between glass and plastic collection tubes. The lack of clinically significant differences in the present study suggests that plastic blood collection tubes can be used in place of glass tubes for a wide variety of coagulation assays using the reagents and instruments described in this study.

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