

Research Article

Impact of centrifugation time reduction in GLP systems

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Article Info

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Keywords

centrifugation time, fully automated, GLP systems, turnaround time

Abstract

Background: Centrifugation of specimens is an important pre-analytical process that significantly impacts turnaround time, enabling clinicians to diagnose, treat, and monitor patients more effectively. In this study, we verified different combinations of tubes and identified optimal centrifugation settings for chemical and immunological assays.

Methods: We evaluated 40 leftover blood samples and collected them in 4-mL vacutainer and 2-mL vacuette lithium heparin tubes. All the tubes were centrifuged using an Abbott Automation GLP system. The first set of samples was centrifuged according to the manufacturer's guidelines. The second and third sets of samples were centrifuged at 2700×g for 7 and 5 min, respectively. All samples were analyzed through 30 chemical and 9 immunological assays on Alinity ci analyzers. The allowable total error, paired t-test, slope, intercept, and correlation coefficient (R) were used to determine the significance of differences in the first set of samples.

Results: Centrifugations for 7 and 5 min at 2700×g were within the acceptable range from the manufacturer's protocol after testing 39 assays in both vacutainer and vacuette lithium heparin tubes, except for LDH that was centrifuged for 5 min at 2700×g.

Conclusions: Shorter centrifugation times (7 min at 2700×g) can be used for different tube combinations. Centrifugation for 5 min at 2700×g affected LDH assays in vacutainer and vacuette lithium heparin tubes.

Introduction

Clinical chemistry laboratories use fully automated GLP systems to reduce human errors and to ensure that the pre-analysis, analysis, and post-analysis processes are accurate, precise, fast, and safe. GLP systems can manage samples more efficiently by monitoring various processes, including centrifugation, decappers, aliquoters, and automatic sample transport to the analyzer via a tracking system. During post-analysis, recappers and refrigerators are used for sample management and storage.

A turnaround time (TAT) is generally employed to assess the efficiency of laboratory management, from sample receipt to reporting the results to the service recipient. Heparinized plasma is used instead of serum for performing clinical, chemical, and immunological tests to reduce the waiting time for analysis results and improve customer satisfaction. This is because lithium heparin tubes prevent blood clotting and can be immediately centrifuged. As lithium heparin inhibits the activity of thrombin III or anti-thrombin III, it helps prevent blood clotting by reducing the breakdown of red blood cells (RBCs) outside the body [1-3]. Centrifugation is an important pre-analytical process that affects the TAT and quality of assays by separating the serum and plasma from RBCs and other components, such as platelets and fibrin.

Previous studies on the effect of centrifugation on chemical test results demonstrated that the test results are not affected by a reduction in the centrifugation time [4-6]. Most of these studies have been performed using tubes from the same manufacturer but with different types of samples, such as serum or plasma. The present study aims to evaluate the effective g-force and the optimal time required for processing two types of test tubes that will be implemented in routine: 4-mL plastic vacutainer lithium heparin tubes from Becton Dickinson and 2-mL plastic vacuette lithium heparin tubes from Greiner Bio-One. The goal is to identify the minimum blood volume necessary for accurate and reliable results, enabling the method's application in patients with limited options for testing and in whom it is difficult to collect blood. The vacutainer tubes should be centrifuged for 10 min at 1000–1300×g in a swinging bucket centrifuge and for 15 min in a fixed-angle centrifuge [7], while the vacuette tubes should be centrifuged for 10–15 min at 1800–2200×g [8]. Notably, the World Health Organization (WHO) also recommends centrifuging plasma for 15 min at 2000–3000×g [9].

Materials and Methods

Sample collection and processing

This study was conducted at the Clinical Chemistry Laboratory of Songklanagarind Hospital, Thailand. The protocol was approved by the Ethics Committee of the Faculty of Medicine, Prince of Songkla University, Thailand (REC. 65-511-5-8).

For this study, we employed 40 leftover lithium heparin blood samples from the analysis conducted at our Clinical Chemistry Laboratory. To reduce the biological variability, we prepared the lithium heparin blood samples for blood group examination by separating them into A, B, AB, and O blood groups. Plasma having hemolysis and lipemia were excluded. We selected a set of normal and abnormal values for the test to provide a distribution of data. Approximately 20 mL of the pooled lithium heparin blood with matching blood groups were collected and mixed to obtain one sample. This sample was then divided into three 4-mL plastic vacutainer lithium heparin tubes (REF 368496) and three 2-mL plastic vacuette lithium heparin tubes (REF 454237). All the tubes were centrifuged using an Abbott Automated GLP System (Hamburg, Germany). The first set of samples (in both vacutainer and vacuette tubes) was centrifuged according to the manufacturer's guidelines (10 min at 1300×g for vacutainer tubes and 10 min at 2200×g for vacuette tubes). The second set was centrifuged for 7 min at 2700×g, and the third set was centrifuged for 5 min at 2700×g at room temperature. Subsequently, all the samples were analyzed based on 30 chemical and 9 immunological parameters, including hemolysis (HEM), icterus (ICT), and lipemia (LIP), on Alinity ci analyzers (Abbott). All analytical procedures were tested under the same conditions and completed in a time duration of 4 h.

Precision study

The total precision of all assays from internal quality control was evaluated using two levels of commercial control materials. Multichem S plus levels 1 and 3 (lot 13111220) and Multichem IA plus levels 1 and 3 (lot 37209220) were used for the immunology assays (TECHNOPATH Ballina, Co., Tipperary, Ireland). The coefficients of variation (CV) of all assays were within the acceptable criterion (<1/3rd of the total allowable error (TEa) limit), as listed in Table 1.

Table 1: Coefficients of variations (CV) of all assays according to internal quality measurement.

Analyte	Abbreviation	%CV		TEa	Resource
		Level 1	Level 2		
Alpha-Fetoprotein, ng/mL	AFP	3.2	2.71	20%	CLIA
Albumin, g/dL	ALB	0.7	0.57	8%	CLIA
Alkaline phosphatase, U/L	ALP	3.31	2.07	20%	CLIA
Alanine aminotransferase, U/L	ALT	2.83	1.73	6 U/L or 15%	CLIA
Amylase, U/L	AMY	2.37	1.65	20%	CLIA
Aspartate aminotransferase, U/L	AST	1.35	0.8	6 U/L or 15%	CLIA
Direct bilirubin, mg/dL	DBIL	5.06	5.29	20%	WLSH
Total bilirubin, mg/dL	TBIL	4.82	5.2	20%	CLIA
Cancer antigen 125, ng/mL	CA 125	3.37	3.24	20%	CLIA
Cancer antigen 19-9, U/mL	CA 19-9	5.7	5.28	30%	BV
Calcium, mg/dL	CA	1.29	1.05	1 mg/dL	CLIA
Carcinoembryonic antigen, ng/mL	CEA	5.38	3.31	1 ng/mL or 15%	CLIA
Cholesterol, mg/dL	CHOL	1.1	0.72	10%	CLIA
Creatine kinase, U/L	CK	1.37	1	20%	CLIA
Chloride, mmol/L	CL	0.73	0.74	5%	CLIA
Bicarbonate, mmol/L	CO2	5.89	5.59	20%	CLIA
Creatinine, mg/dL	CREA	2.32	0.96	10%	CLIA
High-sensitivity CRP, mg/L	hs-CRP	1.63	1.03	30%	CLIA
C-reactive protein, mg/L	CRP	2	1.29	30%	CLIA
Low-density lipoprotein, mg/dL	LDL	3.37	2.44	20%	CLIA
Ferritin, ng/mL	FER	4.49	4.38	20%	CLIA
Free prostate specific antigen, ng/mL	FPSA	2.99	4.31	30%	BV
Gamma-glutamyl transferase, U/L	GGT	2.98	1.65	5 U/L or 15%	CLIA
Glucose, mg/dL	GLU	0.82	0.75	8%	CLIA
Troponin I, ng/L	TNI	7.08	4.24	0.9 ng/mL or 30%	CLIA
Iron, µmol/L	IRON	2.23	1.46	15%	CLIA
Potassium, mmol/L	K	0.63	0.52	0.3 mmol/L	CLIA
Lactate dehydrogenase, U/L	LDH	4.77	1.84	15%	CLIA
Lipase, U/L	LP	3	3.44	20%	BV
Sodium, mmol/L	NA	0.58	0.68	4 mmol/L	CLIA
N-terminal pro B-type natriuretic peptide, pg/mL	NT-proBNP	4.17	3.3	30%	CLIA
Phosphorus, mg/dL	PHOS	1.87	1.34	0.3 mg/dL or 10%	CLIA
Total prostate specific antigen, ng/mL	TPSA	4.99	4.95	0.2 mg/dL or 20%	CLIA
Total protein, g/dL	TP	0.82	0.72	8%	CLIA
Triglyceride, mg/dL	TG	1.27	1.39	15%	CLIA
Unsaturated iron-binding capacity, µmol/L	UIBC	5.85	3.58	20%	AAB
High-density lipoprotein, mg/dL	HDL	1.97	1.92	6 mg/dL or 20%	CLIA
Urea nitrogen, mg/dL	BUN	3	1.69	2 mg/dL or 9%	CLIA
Uric acid, mg/dL	URIC	0.95	0.79	10%	CLIA

The coefficients of variation (CV) of all assays were under 1/3rd of the TEa limit derived from CLIA (Clinical Laboratory Improvement Amendments), WLSH (Wisconsin State Laboratory of Hygiene), and BV (2004 update of the Spanish Society of Clinical Chemistry and Molecular Pathology (SEQC) table of Desirable Quality Specifications based on Biological Variation).

Statistical analysis

Statistical analyses were performed using Excel version 2021, SPSS version 23, and EP Evaluator version 12.3.0.2 (Data Innovations, South Burlington, USA). All results are presented as mean (SD), slope, intercepts, correlation, and paired t-test. The level of statistical significance was set at $P < 0.05$ for the assay with a normal distribution and the Wilcoxon signed-rank test for a non-normal distribution by using the Kolmogorov–Smirnov test to assess the distribution. The results obtained after performing centrifugation for 7 and 5 min at 2700×g using each tube type

were compared. The percentage difference (% difference) of the assay and the number of samples were calculated. For the results to be acceptable, the % difference for each test must be $> 95\%$ within the acceptable TEa limit.

Results

The chemical and immunological results obtained using each tube type are summarized in Table 2. Results obtained after centrifuging each sample for 7 and 5 min at 2700×g, according to the manufacturer's guidelines, are compared.

Table 2: Comparison of chemical and immunological results obtained using different centrifugation settings and tube types.

Analyte	Vacutainer tubes			Vacuette tubes		
	1300×g/10 min	2700×g/7 min	2700×g/5 min	2200×g/10 min	2700×g/7 min	2700×g/5 min
AFP	265.0 (793.12)	270.0 (830.94)	274.6 (837.48)	266.4 (821.86)	265.3 (807.53)	268.3 (815.03)
ALB	4.28 (0.37)	4.29 (0.38) ¹	4.31 (0.39) ¹	4.26 (0.37)	4.30 (0.39) ¹	4.30 (0.39) ¹
ALP	80.5 (30.39)	80.9 (30.34)	80.8 (30.61)	80.1 (30.90)	80.1 (30.86)	82.9 (30.74) ¹
ALT	34.3 (24.32)	34.1 (24.57)	34.3 (24.48)	34.0 (24.28)	34.1 (24.05)	34.0 (24.15)
AMY	101.9 (40.53)	102.0 (40.79)	101.8 (40.58)	101.7 (40.27)	101.7 (40.56)	101.7 (39.99)
AST	28.6 (18.90)	28.4 (18.84) ¹	29.1 (19.0)	27.8 (18.41)	28.2 (18.55) ¹	28.9 (18.77)
DBIL	0.37 (0.41)	0.36 (0.41) ¹	0.37 (0.41)	0.37 (0.41)	0.36 (0.41)	0.37 (0.40) ¹
TBIL	0.84 (0.68)	0.83 (0.68)	0.85 (0.69) ¹	0.84 (0.68)	0.82 (0.67) ¹	0.82 (0.68) ¹
CA 125	116.8 (330.82)	117.9 (339.72)	118.7 (344.53)	116.6 (333.15)	116.8 (329.40)	119.3 (342.05) ¹
CA 19-9	68.3 (118.38)	68.3 (119.03)	68.9 (121.1)	68.2 (118.53)	65.4 (112.86)	69.2 (123.88)
CA	8.88 (0.45)	8.87 (0.43)	8.85 (0.44) ¹	8.85 (0.45)	8.86 (0.43)	8.84 (0.44)
CEA	21.9 (32.48)	20.9 (31.05)	21.3 (32.17)	20.7 (30.39)	20.5 (30.21)	20.5 (30.16)
CHOL	194.3 (41.40)	194.8 (41.57)	196.4 (42.22) ¹	193.8 (41.09)	195.8 (42.79) ¹	196.3 (42.33) ¹
CK	511.3 (1146.08)	509.0 (1141.13)	514.2 (1161.38)	512.3 (1150.42)	513.0 (1154.32)	514.1 (1160.94)
CL	106.0 (2.89)	105.9 (3.00) ¹	105.9 (3.01) ¹	106.0 (3.02)	106.0 (3.01)	106.0 (2.99)
CO2	19.4 (1.76)	19.4 (1.80)	19.3 (1.78)	19.1 (1.70)	19.0 (1.60)	19.0 (1.65)
CREA	1.22 (1.14)	1.23 (1.14) ¹	1.23 (1.13) ¹	1.23 (1.14)	1.23 (1.13)	1.23 (1.14)
hs-CRP	28.5 (19.09)	28.4 (19.15)	28.4 (19.12)	28.4 (19.04)	28.4 (19.04)	28.6 (19.12)
CRP	30.8 (24.11)	30.8 (24.15)	30.8 (24.03)	30.8 (24.18)	30.7 (24.21)	31.0 (24.11)
LDL	122.4 (36.76)	122.5 (36.56)	123.4 (37.14) ¹	122.0 (36.52)	122.7 (37.06) ¹	123.1 (36.97) ¹
FER	1339.4 (1798.38)	1342.5 (1807.55)	1314.7 (1710.85)	1298.6 (1699.40)	1321.2 (1731.15)	1324.7 (1759.94)
FPSA	0.74 (0.85)	0.75 (0.85)	0.75 (0.85)	0.74 (0.84)	0.74 (0.86)	0.75 (0.86)
GGT	87.4 (62.51)	87.0 (62.50)	86.9 (62.1) ¹	86.5 (63.0)	86.4 (62.82)	87.0 (62.57)
GLU	101.5 (19.96)	101.1 (19.86) ¹	101.2 (20.11) ¹	101.1 (19.96)	100.8 (20.04)	100.7 (20.14) ¹
TNI	381.2 (858.64)	384.7 (871.41)	381.7 (878.91) ¹	383.0 (883.31)	385.4 (889.38)	377.6 (864.60)
IRON	13.5 (4.08)	13.5 (4.09)	13.5 (4.13)	13.5 (4.09)	13.5 (4.04)	13.6 (4.10)
K	4.22 (0.54)	4.24 (0.54)	4.22 (0.54)	4.23 (0.53)	4.23 (0.53)	4.23 (0.55)
LDH	427.1 (184.32)	425.1 (179.87)	418.4 (199.54) ^{1, 2}	392.8 (182.0)	391.5 (182.48)	417.4 (194.20) ^{1, 2}
LP	54.2 (26.17)	54.4 (26.13)	54.3 (26.26)	54.1 (26.09)	54.2 (26.22)	54.2 (26.17)
NA	139.9 (2.28)	139.8 (2.35)	139.7 (2.40) ¹	139.6 (2.39)	139.7 (2.45)	139.6 (2.45)
NT-proBNP	4487.9 (5568.05)	4406.4 (5396.71) ¹	4389.7 (5398.91) ¹	4389.5 (5423.77)	4368.9 (5362.38)	4397.0 (5532.67)

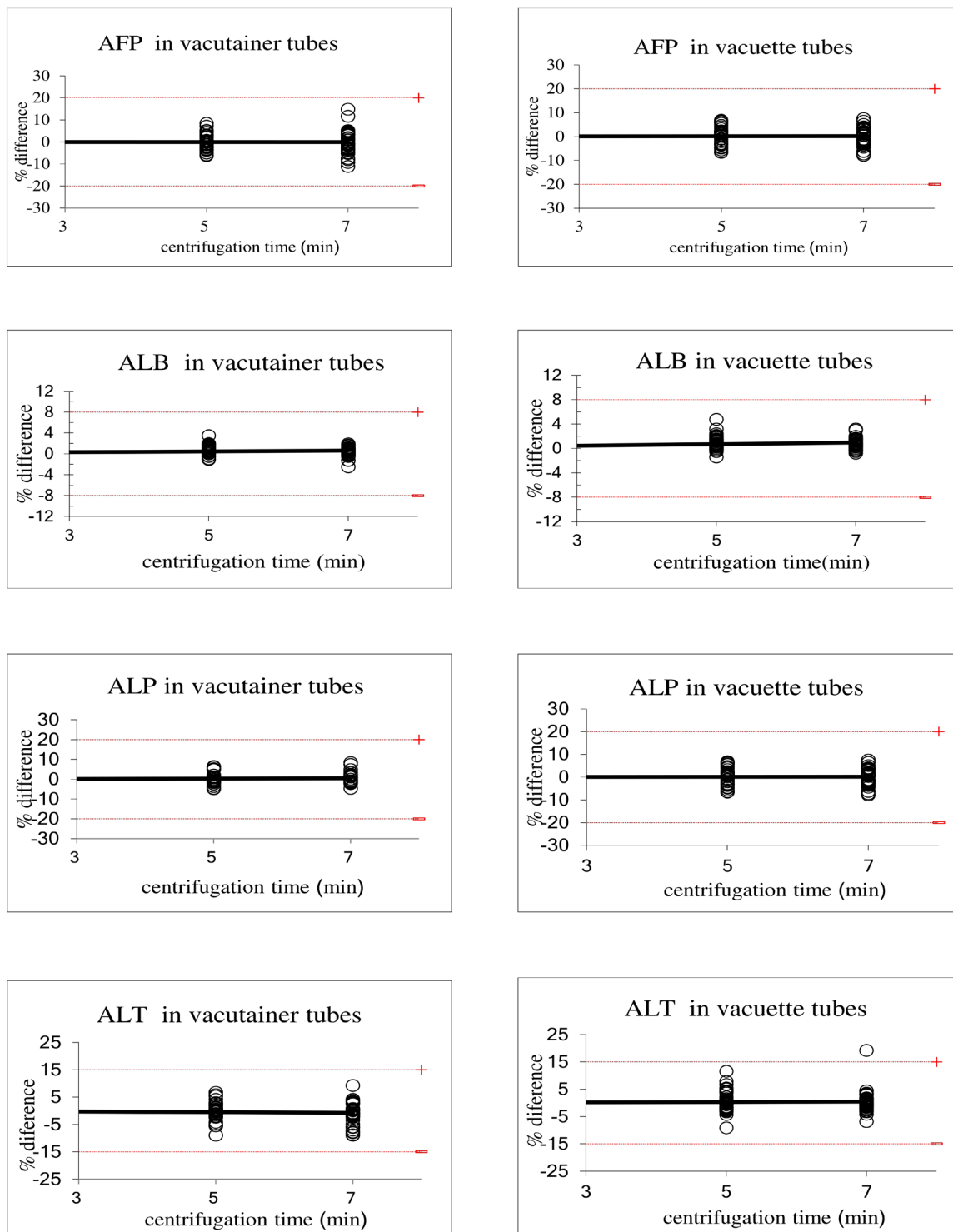
Analyte	Vacutainer tubes			Vacurette tubes		
	1300×g/10 min	2700×g/7 min	2700×g/5 min	2200×g/10 min	2700×g/7 min	2700×g/5 min
PHOS	3.2 (0.40)	3.2 (0.40)	3.2 (0.40)	3.2 (0.40)	3.2 (0.40)	3.1 (0.40) ¹
TPSA	4.0 (7.37)	4.1 (7.53)	4.0 (7.24)	4.0 (7.10)	4.1 (7.55)	4.1 (7.58)
TP	7.63 (0.53)	7.64 (0.54)	7.70 (0.58) ¹	7.63 (0.52)	7.68 (0.57) ¹	7.70 (0.57) ¹
TG	134.8 (95.36)	135.3 (97.01)	133.3 (94.57) ¹	131.6 (93.63)	131.9 (94.32)	130.1 (93.38) ¹
UIBC	38.9 (9.43)	39.0 (9.42)	39.3 (9.63) ¹	38.8 (9.33)	38.9 (9.45)	39.4 (9.69) ¹
HDL	53.6 (11.79)	53.8 (11.81)	54.6 (12.61) ¹	53.5 (11.89)	54.8 (12.90) ¹	54.8 (12.65) ¹
BUN	16.8 (10.7)	16.8 (10.78)	16.9 (10.65) ¹	16.6 (10.76)	16.7 (10.65)	16.7 (10.70) ¹
URIC	5.6 (1.38)	5.6 (1.38) ¹	5.6 (1.38)	5.7 (1.37)	5.7 (1.38)	5.6 (1.39) ¹

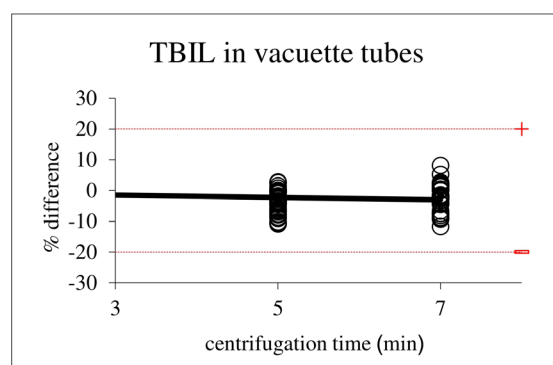
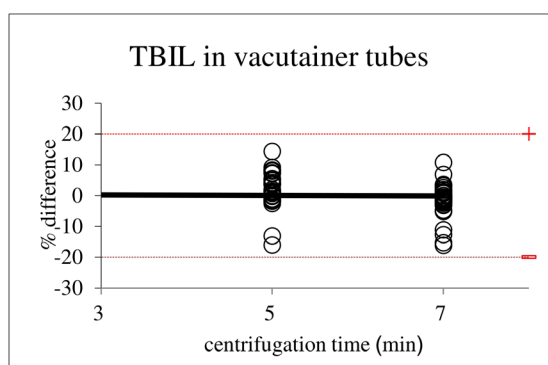
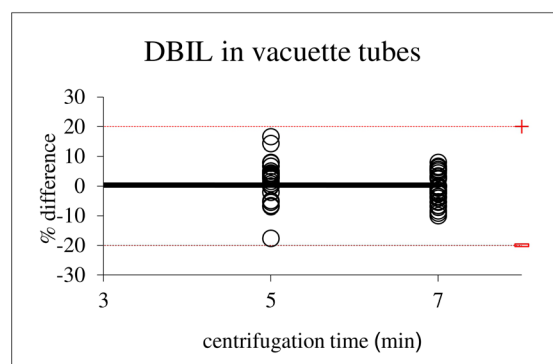
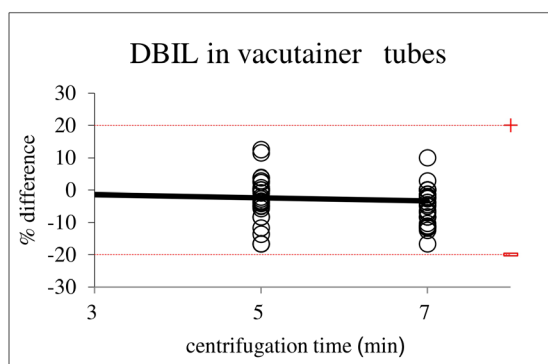
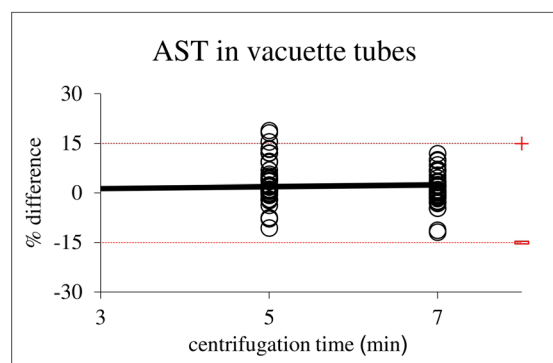
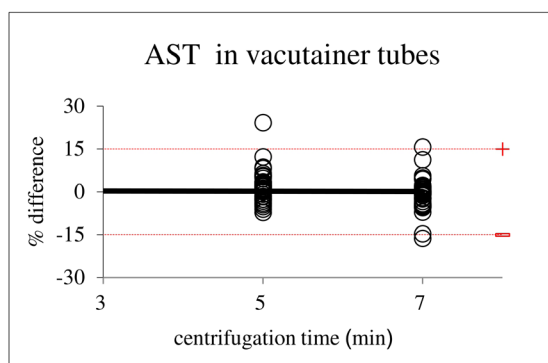
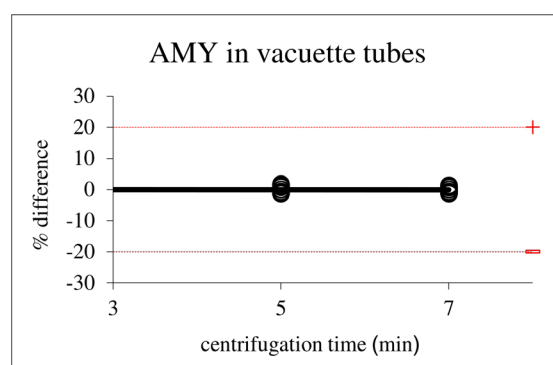
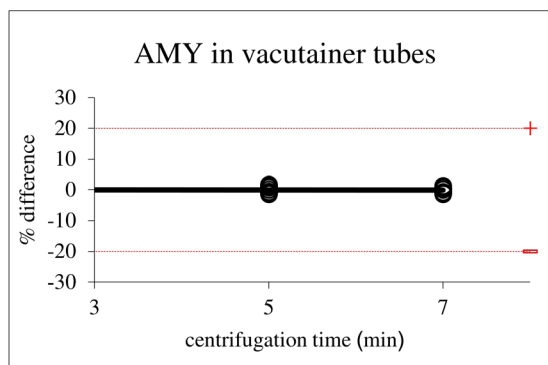
Data are presented as mean, and standard deviation (SD); ¹P<0.05 was considered statistically significant and ²>5% of individuals exceed the allowable error limit.

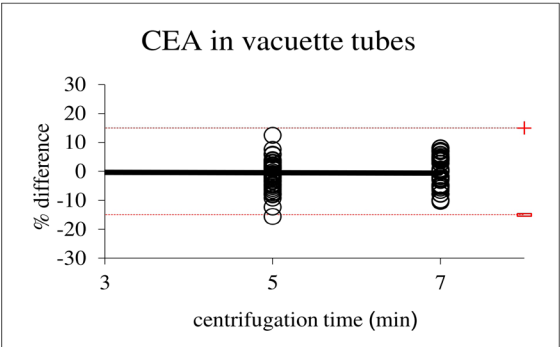
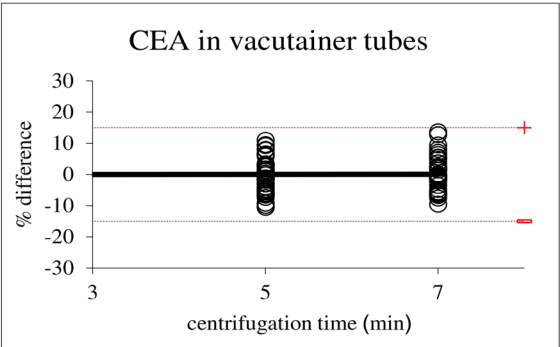
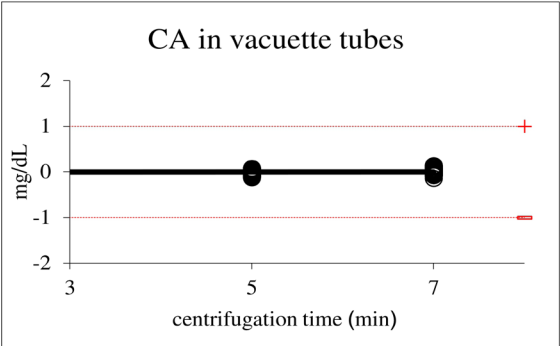
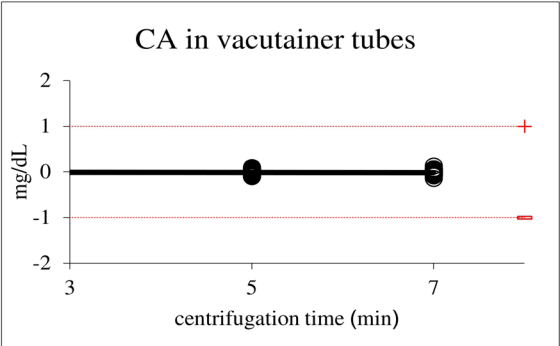
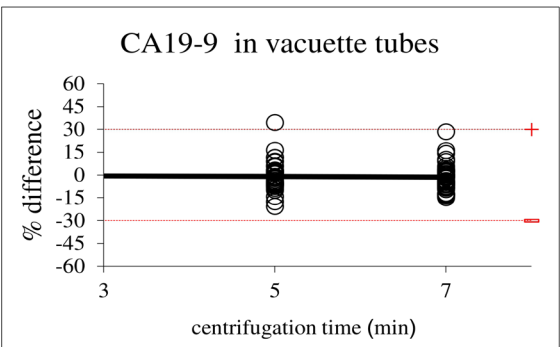
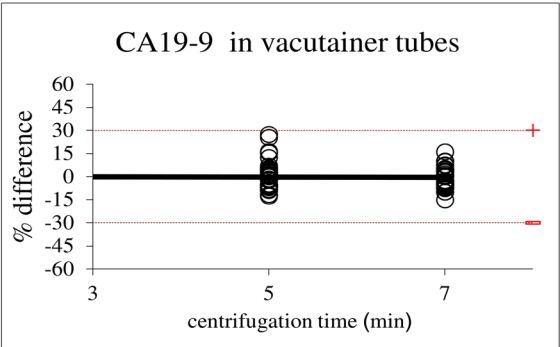
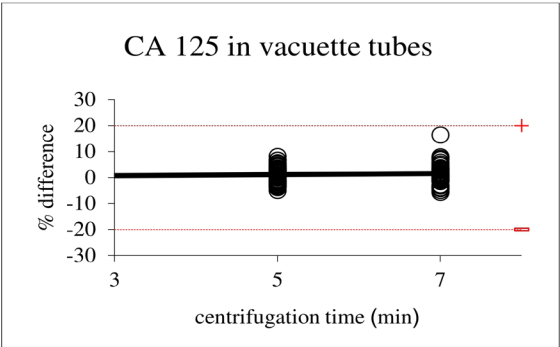
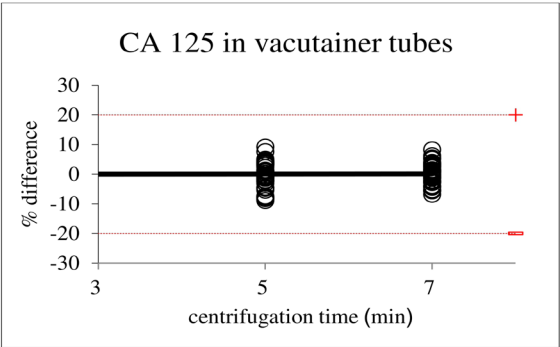
Samples in vacutainer tubes centrifuged for 7 min at 2700×g demonstrated statistical differences in the ALB, AST, DBIL, CL, CREA, GLU, LDH, NT-proBNP and URIC assay results, while samples in vacurette tubes demonstrated statistical differences in the ALB, TBIL, CHOL, LDL, TP, HDL and BUN assay results. Although the test results were different, they were within the acceptable range of TEa, as shown in Figure 1. For samples in vacutainer tubes centrifuged for 5 min at 2700×g, the ALB,

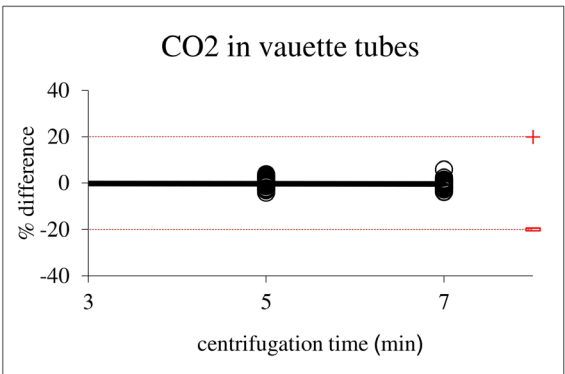
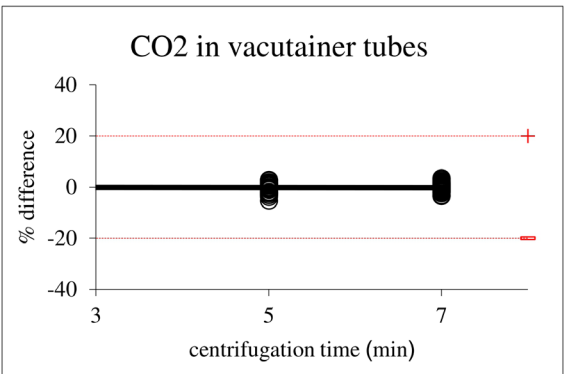
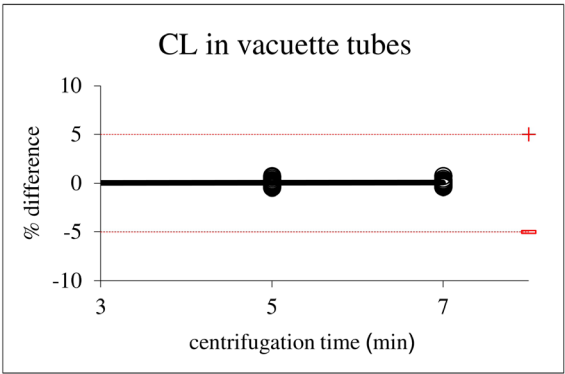
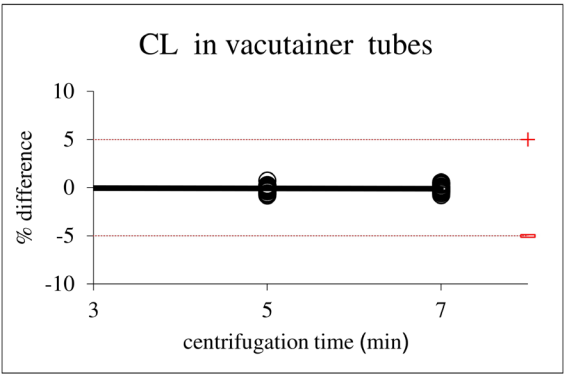
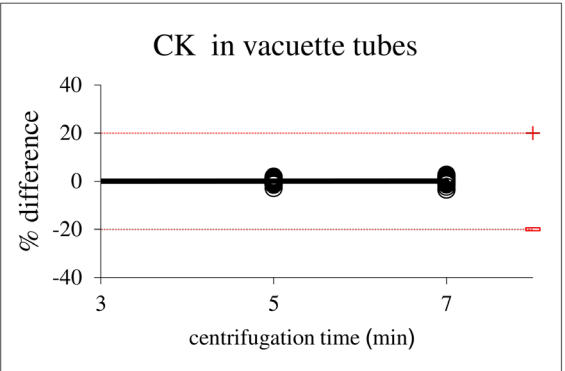
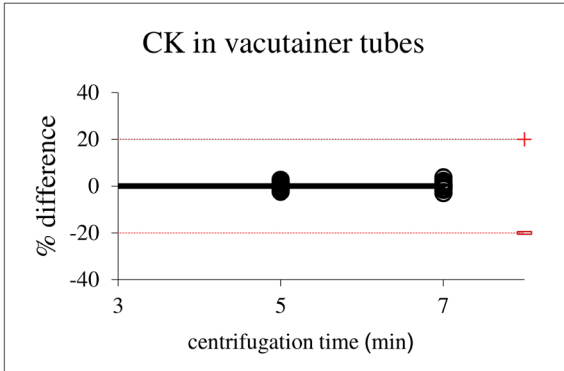
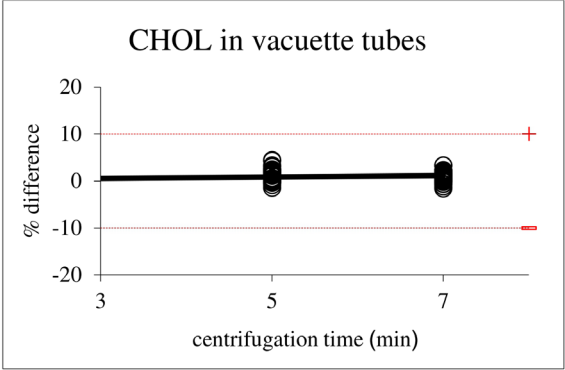
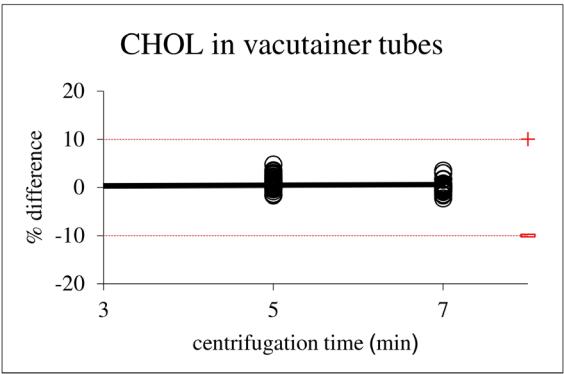
TBIL, CA, CHOL, CL, CREA, LDL, GGT, GLU, TNI, NA, NT-proBNP, TP, TG, UIBC, HDL, BUN, and URIC assay results were statistically different, as were the ALB, ALP, AST, DBIL, TBIL, CA, CHOL, CL, CREA, CA125, CHOL, LDL, GGT, GLU, PHOS, TP, TG, UIBC, HDL, BUN and URIC assay results in vacurette tubes. All test results were within the acceptable range of TEa, except for LDH, which demonstrated a percentage difference of <95%, as shown in Figure 1.

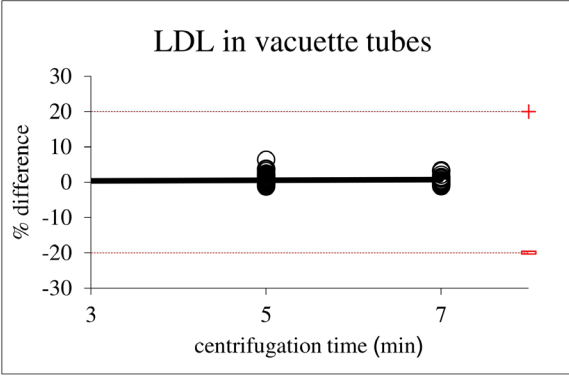
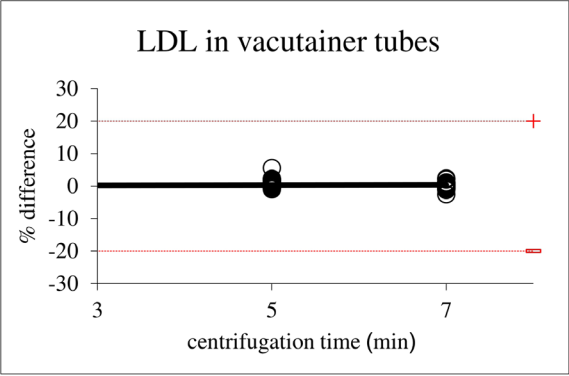
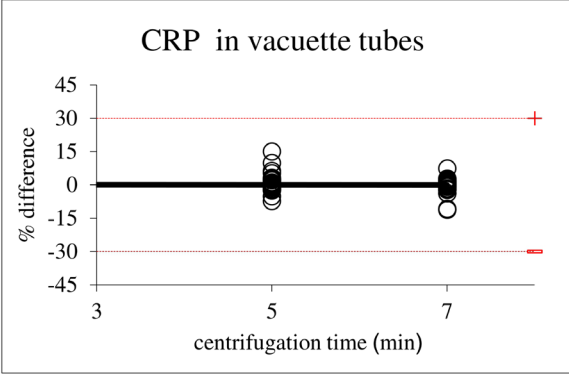
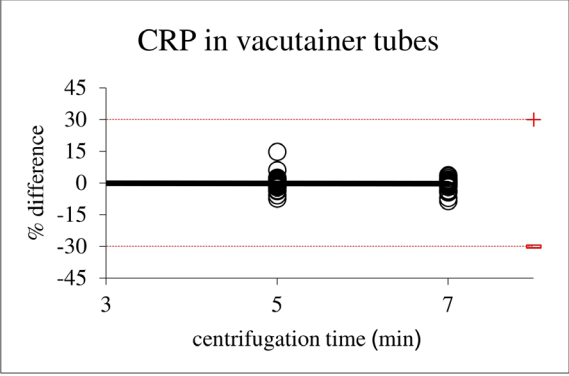
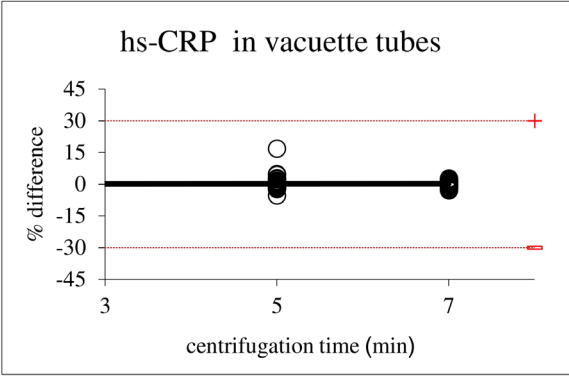
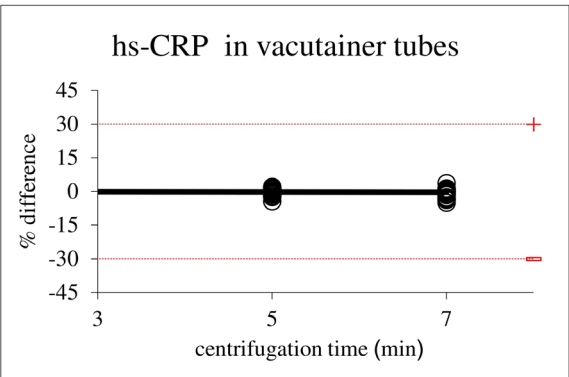
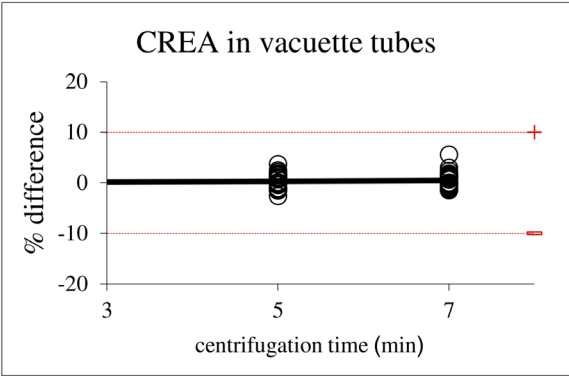
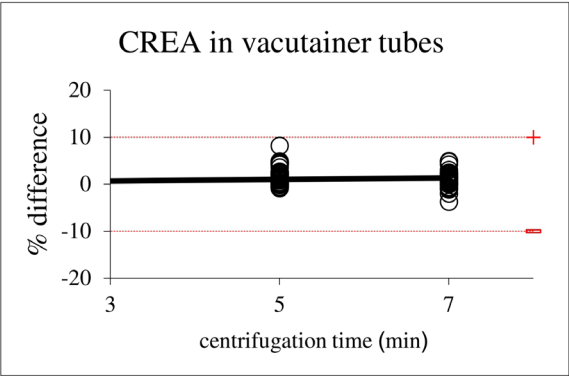
Figure 1: Percentage differences of assays (N=40) after centrifuging the samples in each tube for 7 and 5 min at 2700×g, according to the manufacturer's recommendations.

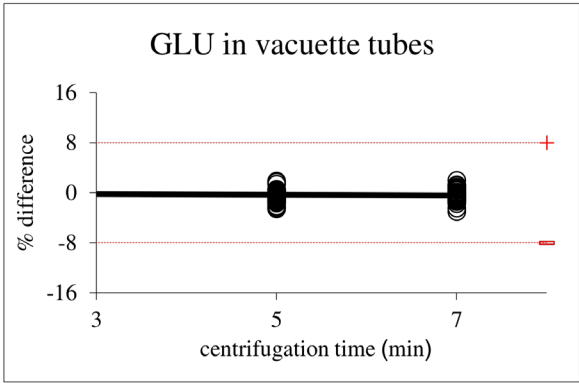
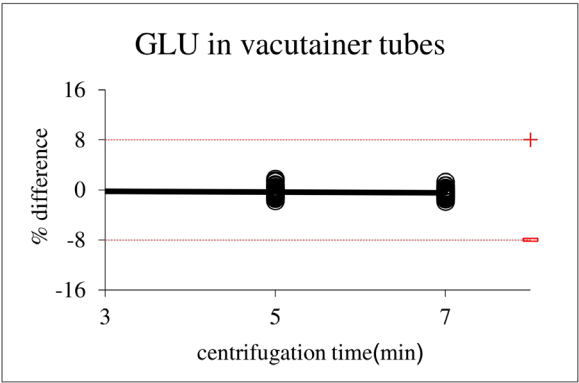
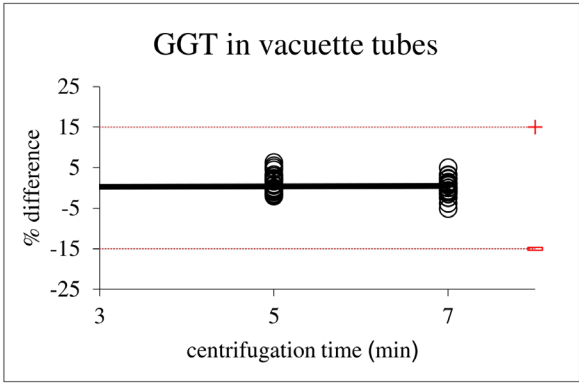
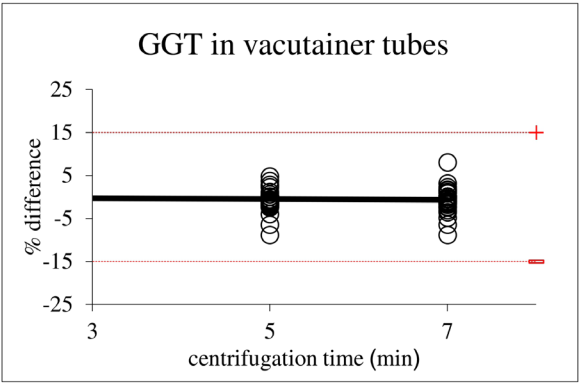
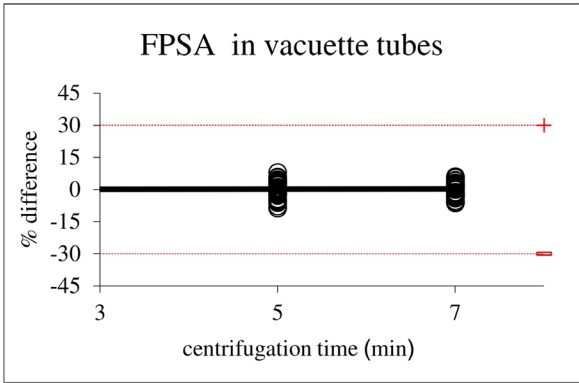
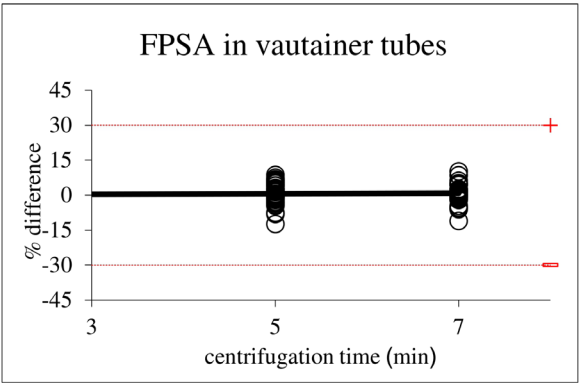
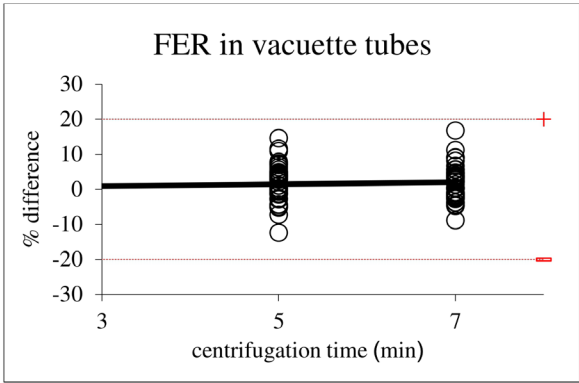
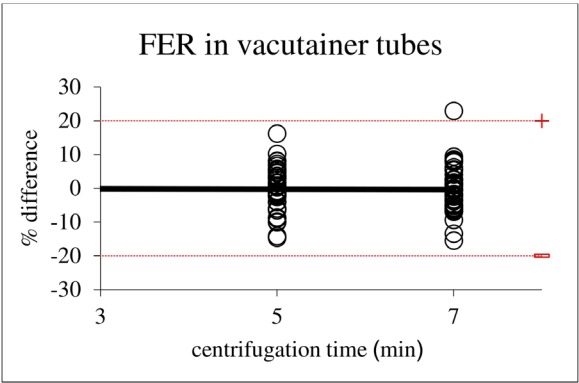


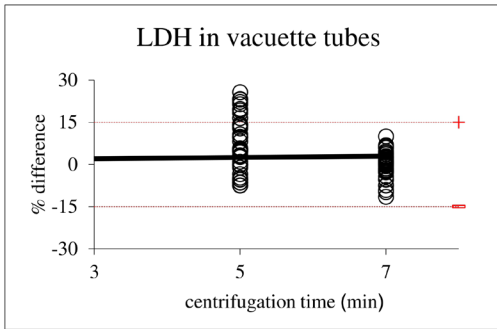
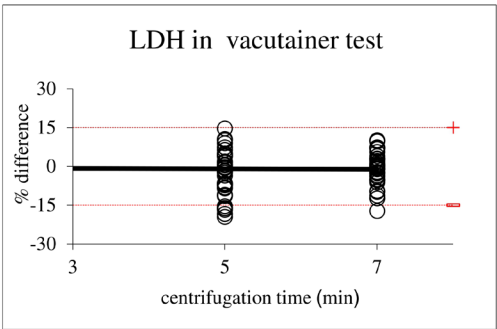
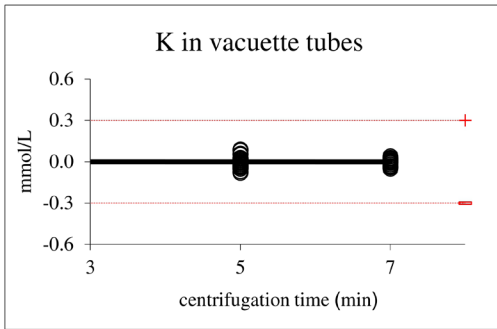
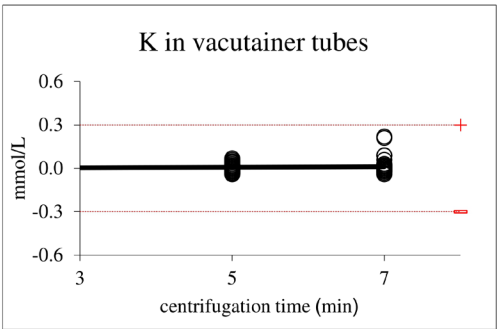
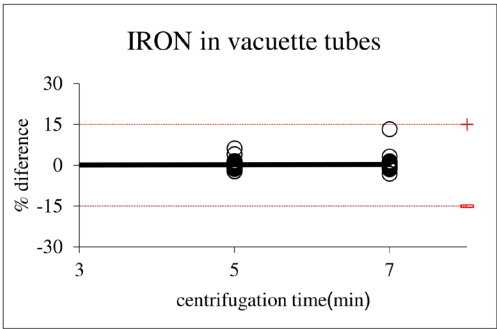
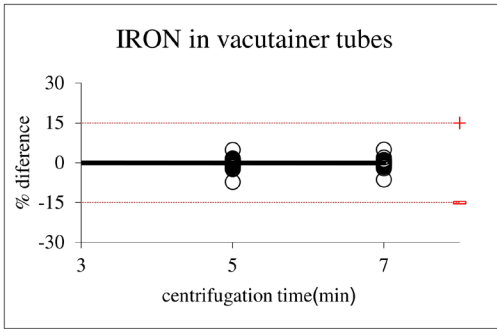
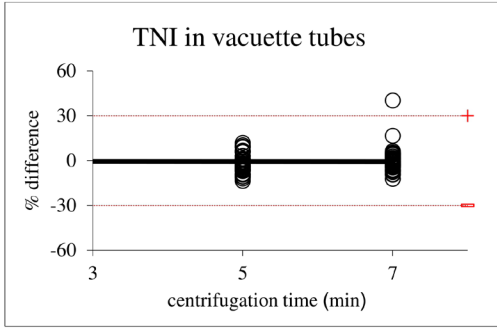
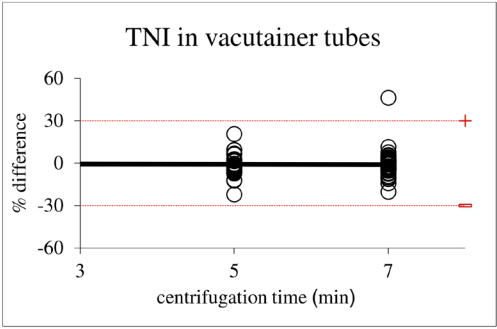


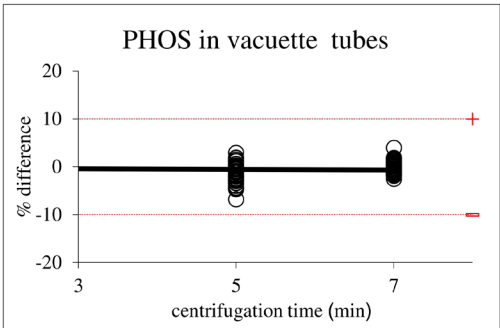
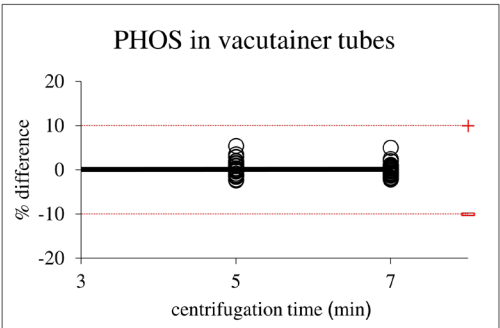
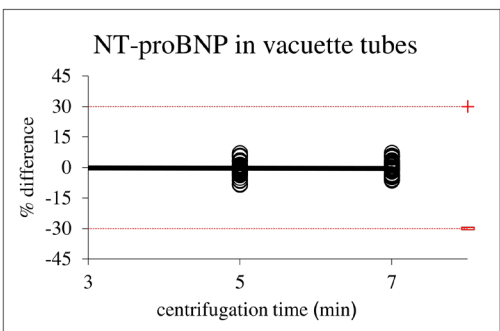
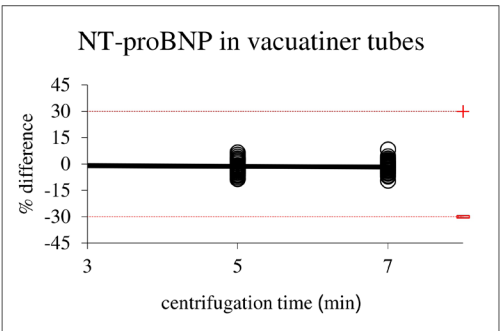
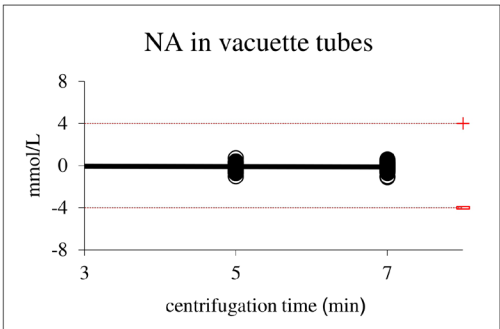
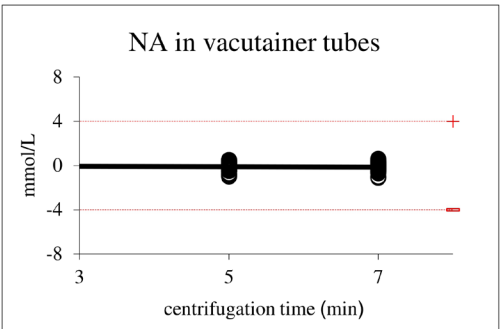
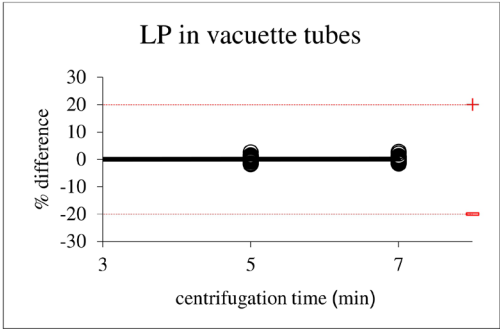
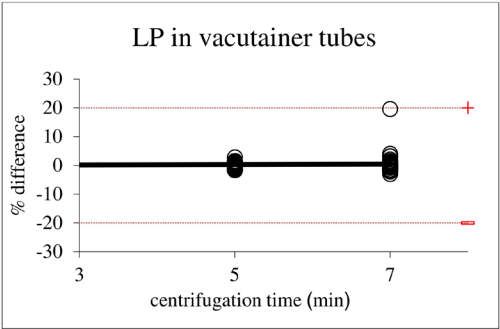


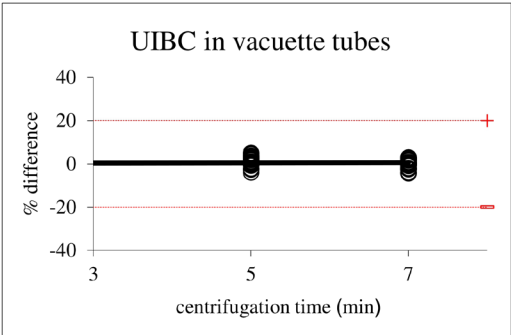
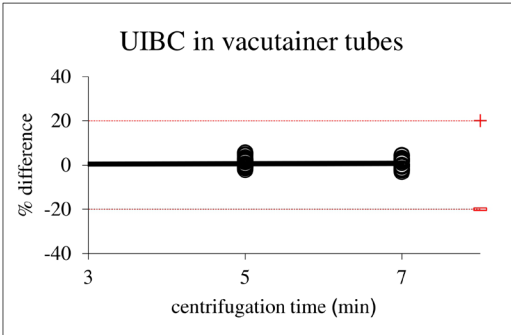
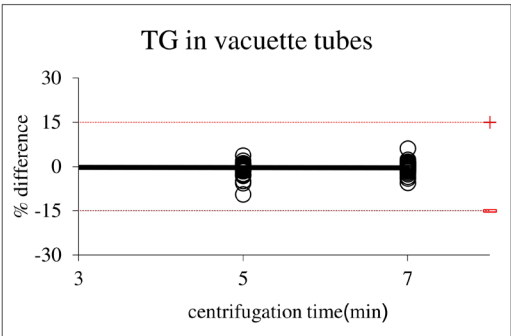
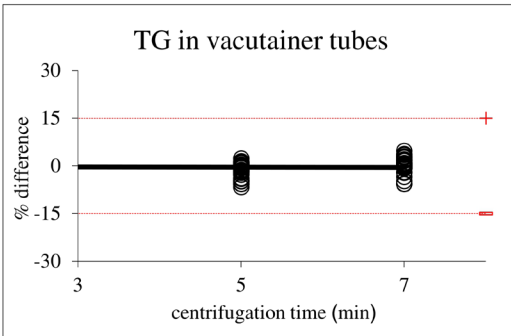
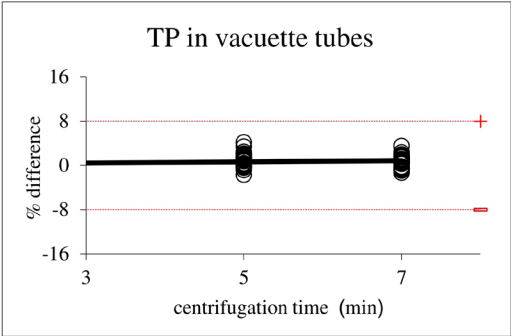
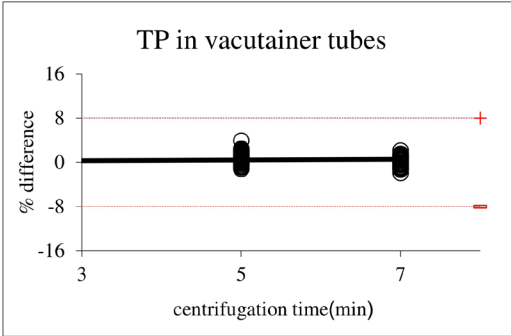
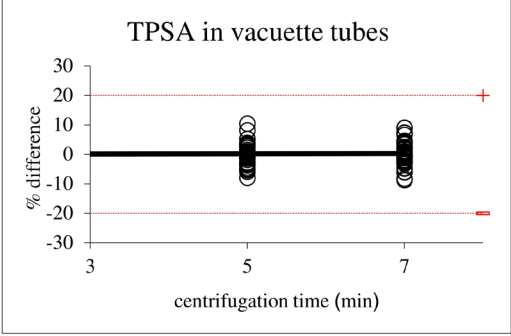
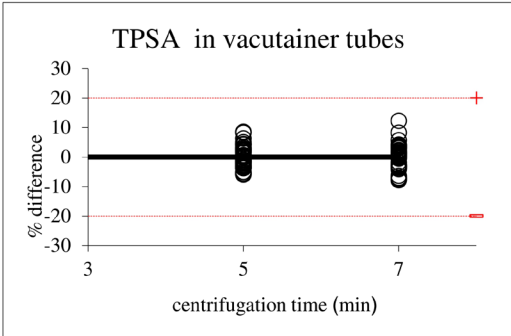


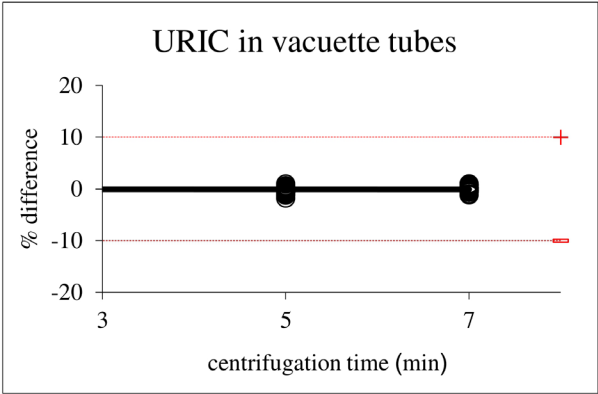
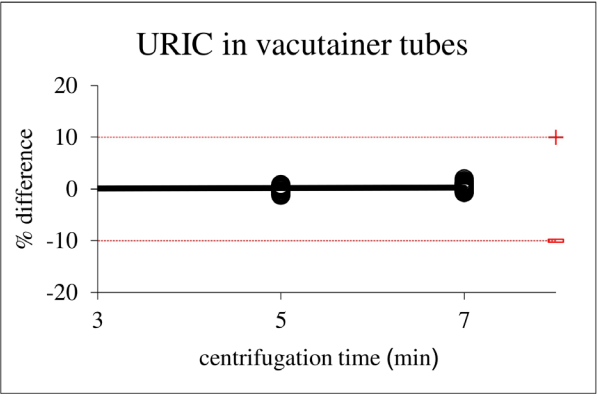
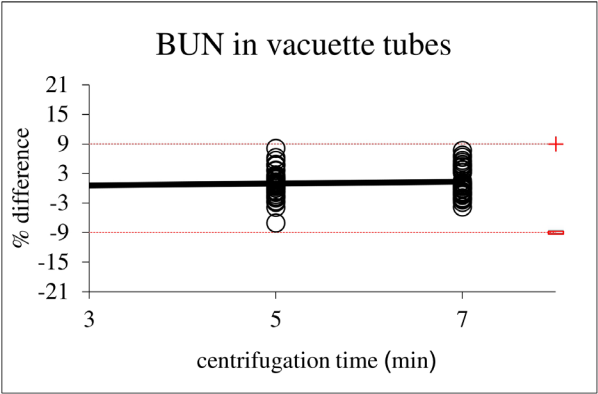
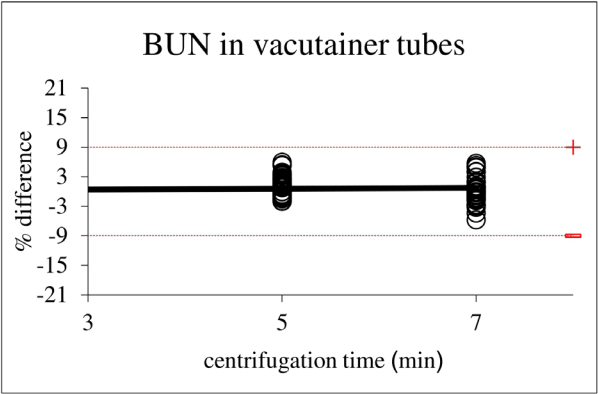
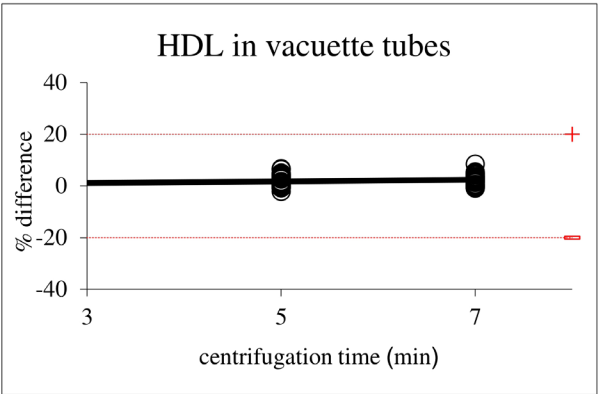
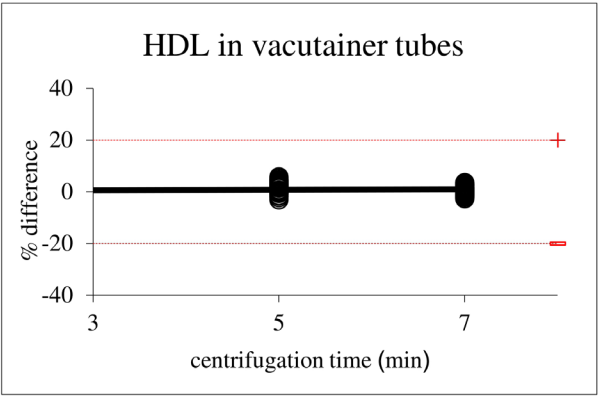












The percentage differences of each test must be >95% within acceptable TEa limits except LDH in vacutainer and vacuette tubes at 5 min.

The index results for hemolysis (HEM), icterus (ICT), and lipemia (LIP) cannot be interpreted since most of the measured values were below the lowest detection limit of the assay. The Passing–Bablok regression analysis results showed that the slope and intercept lie in the 95% confidence interval (95%

CI) (indicated with parentheses. Furthermore, the correlation coefficient obtained using vacutainer and vacuette tubes for centrifugation at 7 and 5 min at 2700×g correlated well with the acceptable limit (>0.9). The corresponding results are listed in Tables 3–6.

Table 3: Comparison of chemical and immunological assays after centrifugation at 2700×g for 7 min and 1300×g for 10 min on vacutainer tubes.

Tests	Slope (95% CI)	Intercept (95% CI)	Correlation coefficient (AL)	Correlation
AFP	0.981 (0.950 to 0.999)	0.0816 (-0.0135 to 0.1691)	0.9962	Acceptable
ALB	1.022 (1.000 to 1.061)	-0.079 (-0.235 to 0.010)	0.9948	Acceptable
ALP	1.000 (1.000 to 1.016)	0.000 (-1.057 to 0.000)	0.9986	Acceptable
ALT	1.011 (0.998 to 1.024)	-0.552 (-0.900 to -0.141)	0.9994	Acceptable
AMY	1.000 (1.000 to 1.000)	0.0 (0.0 to 0.0)	0.9999	Acceptable
AST	1.008 (0.985 to 1.038)	-0.229 (-0.908 to 0.215)	0.9969	Acceptable
DBIL	1.000 (0.995 to 1.000)	-0.010 (-0.010 to -0.007)	0.9995	Acceptable
TBIL	1.000 (1.000 to 1.000)	0.000 (0.000 to 0.000)	1.0000	Acceptable
CA 125	0.999 (0.988 to 1.015)	0.3592 (-0.5550 to 1.0302)	0.9999	Acceptable
CA 19-9	0.979 (0.952 to 1.007)	-0.0840 (-0.4126 to 0.4198)	0.9986	Acceptable
CA	0.952 (0.917 to 1.000)	0.410 (-0.010 to 0.728)	0.9933	Acceptable
CEA	0.956 (0.934 to 0.993)	0.1833 (-0.0030 to 0.2749)	0.9990	Acceptable
CHOL	1.007 (0.992 to 1.020)	-0.892 (-3.341 to 1.711)	0.9988	Acceptable
CK	1.000 (0.986 to 1.000)	0.0 (0.0 to 2.1)	0.9999	Acceptable
CL	1.026 (1.000 to 1.064)	-2.93 (-6.90 to -0.10)	0.9959	Acceptable
CO2	1.020 (0.960 to 1.091)	-0.41 (-1.85 to 0.75)	0.9837	Acceptable
CREA	1.000 (0.995 to 1.000)	0.010 (0.010 to 0.013)	0.9999	Acceptable
hs-CRP	1.001 (0.995 to 1.006)	-0.056 (-0.166 to 0.055)	0.9998	Acceptable
CRP	1.004 (0.996 to 1.012)	-0.060 (-0.349 to 0.033)	0.9998	Acceptable
LDL	0.996 (0.987 to 1.004)	0.389 (-0.484 to 1.532)	0.9996	Acceptable
FER	0.988 (0.966 to 1.019)	2.9823 (-11.220 to 16.4556)	0.9988	Acceptable
FPSA	1.005 (0.996 to 1.016)	0.0006 (-0.0018 to 0.0045)	0.9996	Acceptable
GGT	1.000 (0.996 to 1.000)	0.0 (0.0 to 0.1)	0.9997	Acceptable
GLU	0.997 (0.986 to 1.005)	-0.06 (-0.98 to 0.92)	0.9995	Acceptable
HEM	1.250 (1.000 to 1.500)	-0.250 (-1.500 to 0.500)	0.9624	Not interpret
ICT	1.000 (1.000 to 1.000)	0.00 (0.00 to 0.00)	0.9466	Not interpret
TNI	1.001 (0.981 to 1.019)	-0.253 (-1.138 to 0.440)	0.9996	Acceptable
IRON	1.000 (0.991 to 1.007)	0.000 (-0.082 to 0.106)	0.9988	Acceptable
K	1.000 (0.993 to 1.036)	0.000 (-0.143 to 0.029)	0.9947	Acceptable
LIP	0.900 (0.769 to 1.000)	-4.000 (-5.000 to -2.269)	0.9568	Not interpret
LDH	1.032 (0.938 to 1.146)	-16.2 (-66.1 to 14.7)	0.9826	Acceptable
LP	1.000 (0.991 to 1.007)	0.0009 (-0.3491 to 0.4433)	0.9981	Acceptable
NA	1.038 (0.985 to 1.100)	-5.43 (-14.11 to 2.07)	0.9872	Acceptable
NT-proBNP	0.985 (0.970 to 0.998)	3.728 (-11.824 to 16.353)	0.9996	Acceptable
PHOS	1.000 (0.968 to 1.036)	0.000 (-0.128 to 0.098)	0.9949	Acceptable
TPSA	1.010 (0.992 to 1.030)	-0.0034 (-0.0162 to 0.0044)	0.9986	Acceptable

TP	1.023 (0.976 to 1.063)	-0.144 (-0.459 to 0.205)	0.9922	Acceptable
TG	1.014 (0.998 to 1.026)	-1.41 (-2.75 to 0.44)	0.9994	Acceptable
UIBC	1.000 (1.000 to 1.034)	0.000 (-1.303 to 0.000)	0.9972	Acceptable
HDL	1.000 (0.978 to 1.022)	0.150 (-0.966 to 1.419)	0.9978	Acceptable
BUN	1.000 (0.985 to 1.008)	0.000 (-0.126 to 0.253)	0.9996	Acceptable
URIC	1.000 (0.991 to 1.008)	0.020 (-0.028 to 0.063)	0.9997	Acceptable

The 95% confidence interval (95% CI) for the slope and intercept is indicated with parentheses. Results cannot be interpreted because most measured values are below the lowest detection limit of the assay.

Table 4: Comparison of chemical and immunological assays after centrifugation at 2700×g for 5 min and 1300×g for 10 min on vacutainer tubes.

Tests	Slope (95% CI)	Intercept (95% CI)	Correlation coefficient (AL)	Correlation
AFP	1.001 (0.984 to 1.034)	-0.0022 (-0.1145 to 0.0463)	0.9994	Acceptable
ALB	1.081 (1.038 to 1.119)	-0.301 (-0.475 to -0.125)	0.9950	Acceptable
ALP	1.000 (1.000 to 1.027)	0.000 (-2.446 to 0.000)	0.9978	Acceptable
ALT	1.005 (0.995 to 1.014)	-0.165 (-0.470 to 0.168)	0.9996	Acceptable
AMY	1.000 (1.000 to 1.000)	0.0 (0.0 to 0.0)	0.9998	Acceptable
AST	1.026 (1.000 to 1.057)	-0.272 (-1.062 to 0.300)	0.9978	Acceptable
DBIL	1.000 (1.000 to 1.033)	0.000 (-0.011 to 0.000)	0.9993	Acceptable
TBIL	1.000 (0.993 to 1.026)	0.010 (-0.011 to 0.015)	0.9987	Acceptable
CA 125	1.004 (0.980 to 1.037)	-0.0108 (-0.8573 to 0.9488)	0.9999	Acceptable
CA 19-9	0.975 (0.935 to 1.032)	0.1540 (-0.8069 to 0.6894)	0.9972	Acceptable
CA	0.992 (0.951 to 1.034)	0.050 (-0.328 to 0.411)	0.9928	Acceptable
CEA	0.972 (0.944 to 1.003)	0.0987 (-0.0297 to 0.2011)	0.9979	Acceptable
CHOL	1.026 (1.004 to 1.045)	-3.155 (-6.989 to 0.926)	0.9981	Acceptable
CK	1.000 (0.996 to 1.005)	0.0 (-0.4 to 0.9)	0.9999	Acceptable
CL	1.040 (1.000 to 1.074)	-4.32 (-7.96 to -0.05)	0.9958	Acceptable
CO2	1.000 (0.942 to 1.074)	0.00 (-1.43 to 1.08)	0.9814	Acceptable
CREA	0.993 (0.979 to 1.000)	0.018 (0.010 to 0.036)	0.9999	Acceptable
hs-CRP	1.000 (0.994 to 1.005)	-0.005 (-0.117 to 0.111)	0.9999	Acceptable
CRP	0.998 (0.992 to 1.006)	0.027 (-0.128 to 0.182)	0.9999	Acceptable
LDL	1.012 (0.998 to 1.025)	-0.844 (-2.215 to 1.018)	0.9993	Acceptable
FER	0.993 (0.965 to 1.037)	4.1364 (-11.1582 to 19.2708)	0.9964	Acceptable
FPSA	1.011 (0.993 to 1.028)	0.0001 (-0.0055 to 0.0030)	0.9993	Acceptable
GGT	1.000 (0.992 to 1.000)	0.0 (0.0 to 0.3)	0.9998	Acceptable
GLU	1.005 (0.993 to 1.022)	-1.12 (-2.82 to 0.13)	0.9991	Acceptable
HEM	1.000 (0.875 to 1.273)	0.000 (-0.864 to 0.438)	0.9561	Not interpret
ICT	1.000 (1.000 to 1.000)	0.00 (0.00 to 0.00)	0.8584	Not interpret
TNI	0.983 (0.963 to 1.004)	-0.197 (-0.793 to 0.600)	0.9985	Acceptable

IRON	1.004 (0.997 to 1.018)	-0.046 (-0.215 to 0.052)	0.9986	Acceptable
K	1.000 (0.988 to 1.026)	0.000 (-0.112 to 0.048)	0.9988	Acceptable
LDH	0.980 (0.936 to 1.031)	7.8 (-13.0 to 29.6)	0.9892	Acceptable
LIP	0.875 (0.750 to 1.000)	-3.750 (-5.000 to -2.000)	0.9715	Not interpret
LP	1.003 (0.995 to 1.010)	-0.0633 (-0.3844 to 0.2892)	0.9998	Acceptable
NA	1.056 (1.000 to 1.108)	-8.04 (-15.23 to -0.20)	0.9890	Acceptable
NT-proBNP	0.976 (0.954 to 0.990)	0.374 (-13.074 to 11.777)	0.9993	Acceptable
PHOS	1.000 (0.962 to 1.046)	0.000 (-0.139 to 0.128)	0.9923	Acceptable
TPSA	0.988 (0.975 to 1.002)	0.0031 (-0.0025 to 0.0145)	0.9988	Acceptable
TP	1.100 (1.061 to 1.146)	-0.689 (-1.052 to -0.392)	0.9925	Acceptable
TG	1.003 (0.993 to 1.018)	-1.18 (-2.86 to 0.47)	0.9994	Acceptable
UIBC	1.002 (1.000 to 1.037)	-0.058 (-1.217 to 0.000)	0.9977	Acceptable
HDL	1.069 (1.039 to 1.106)	-2.835 (-4.786 to -1.115)	0.9970	Acceptable
BUN	0.988 (0.972 to 1.000)	0.394 (0.200 to 0.593)	0.9997	Acceptable
URIC	1.004 (1.000 to 1.011)	-0.013 (-0.058 to 0.005)	0.9998	Acceptable

For the slope and intercept, the 95% confidence interval (95% CI) is indicated with parentheses. Results cannot be interpreted because most measured values are below the lowest detection limit of the assay.

Table 5: Comparison of chemical and immunological assays after centrifugation at 2700×g for 7 min and 2200×g for 10 min on vacuette tubes.

Tests	Slope (95% CI)	Intercept (95% CI)	Correlation coefficient (AL)	Correlation
AFP	1.004 (0.987 to 1.027)	0.0016 (-0.0745 to 0.0470)	0.9994	Acceptable
ALB	1.074 (1.045 to 1.115)	-0.281 (-0.458 to -0.159)	0.9965	Acceptable
ALP	1.000 (1.000 to 1.000)	0.000 (0.000 to 0.000)	0.9993	Acceptable
ALT	0.994 (0.984 to 1.006)	0.194 (-0.147 to 0.571)	0.9991	Acceptable
AMY	1.000 (1.000 to 1.000)	0.0 (0.0 to 0.0)	0.9999	Acceptable
AST	1.023 (1.000 to 1.067)	-0.413 (-1.283 to 0.350)	0.9982	Acceptable
DBIL	1.000 (0.992 to 1.000)	0.000 (0.000 to 0.002)	0.9997	Acceptable
TBIL	1.000 (0.957 to 1.000)	-0.010 (-0.010 to 0.018)	0.9982	Acceptable
CA 125	1.010 (0.990 to 1.033)	-0.0478 (-0.8831 to 0.6025)	0.9999	Acceptable
CA 19-9	0.957 (0.923 to 1.007)	0.4234 (-0.5283 to 1.1428)	0.9984	Acceptable
CA	0.968 (0.912 to 1.018)	0.300 (-0.148 to 0.780)	0.9890	Acceptable
CEA	0.985 (0.970 to 1.004)	0.0574 (-0.0931 to 0.1737)	0.9996	Acceptable
CHOL	1.034 (1.016 to 1.050)	-4.828 (-7.919 to -1.361)	0.9990	Acceptable

CK	1.000 (0.994 to 1.002)	0.0 (-0.3 to 0.9)	1.0000	Acceptable
CL	1.000 (0.963 to 1.014)	0.00 (-1.41 to 3.96)	0.9950	Acceptable
CO2	0.963 (0.897 to 1.000)	0.57 (-0.15 to 1.86)	0.9827	Acceptable
CREA	1.000 (0.993 to 1.000)	0.000 (0.000 to 0.012)	1.0000	Acceptable
hs-CRP	0.998 (0.990 to 1.004)	0.042 (-0.053 to 0.272)	0.9999	Acceptable
CRP	0.998 (0.993 to 1.003)	-0.033 (-0.147 to 0.105)	0.9999	Acceptable
LDL	1.011 (0.998 to 1.026)	-0.822 (-2.342 to 0.424)	0.9993	Acceptable
FER	1.024 (1.000 to 1.039)	-4.7694 (-12.7687 to 6.1334)	0.9992	Acceptable
FPSA	1.005 (0.990 to 1.020)	0.0005 (-0.0031 to 0.0031)	0.9990	Acceptable
GGT	1.000 (0.993 to 1.000)	0.0 (0.0 to 0.4)	0.9999	Acceptable
GLU	1.001 (0.989 to 1.023)	-0.49 (-2.53 to 0.87)	0.9987	Acceptable
HEM	1.051 (0.961 to 1.333)	-1.253 (-3.333 to -0.627)	0.9561	Not interpret
ICT	1.000 (1.000 to 1.000)	0.00 (0.00 to 0.00)	0.9526	Not interpret
TNI	1.006 (0.993 to 1.018)	-0.692 (-1.248 to 0.012)	0.9999	Acceptable
IRON	0.996 (0.983 to 1.000)	0.044 (0.000 to 0.224)	0.9990	Acceptable
K	1.000 (1.000 to 1.025)	0.000 (-0.100 to 0.000)	0.9993	Acceptable
LDH	1.000 (0.968 to 1.033)	1.0 (-12.3 to 12.6)	0.9962	Acceptable
LIP	1.000 (1.000 to 1.000)	0.000 (0.000 to 0.000)	0.9918	Not interpret
LP	1.007 (1.001 to 1.013)	-0.2321 (-0.5360 to 0.0858)	0.9999	Acceptable
NA	1.021 (0.977 to 1.063)	-2.87 (-8.63 to 3.30)	0.9920	Acceptable
NT-proBNP	0.997 (0.985 to 1.012)	0.147 (-13.333 to 10.524)	0.9996	Acceptable
PHOS	1.007 (0.975 to 1.039)	-0.031 (-0.137 to 0.074)	0.9958	Acceptable
TPSA	1.007 (0.987 to 1.027)	-0.0017 (-0.0161 to 0.0094)	0.9986	Acceptable
TP	1.096 (1.061 to 1.142)	-0.678 (-1.037 to -0.425)	0.9940	Acceptable
TG	1.008 (1.000 to 1.018)	-0.48 (-1.52 to 0.40)	0.9997	Acceptable
UIBC	1.000 (1.000 to 1.015)	0.000 (-0.560 to 0.000)	0.9981	Acceptable
HDL	1.065 (1.035 to 1.098)	-2.258 (-4.149 to -0.697)	0.9970	Acceptable
BUN	0.983 (0.964 to 1.000)	0.302 (0.100 to 0.611)	0.9993	Acceptable
URIC	1.009 (1.000 to 1.016)	-0.042 (-0.079 to 0.005)	0.9998	Acceptable

For the slope and intercept, the 95% confidence interval (95% CI) is indicated with parentheses. Results cannot be interpreted because most measured values are below the lowest detection limit of the assay.

Table 6: Comparison of chemical and immunological assays after centrifugation at 2700×g for 5 min and 2200×g for 10 min on vacuette tubes.

Tests	Slope (95% CI)	Intercept (95% CI)	Correlation coefficient (AL)	Correlation
AFP	1.003 (0.989 to 1.044)	0.0223 (-0.1137 to 0.0723)	0.9981	Acceptable
ALB	1.074 (1.042 to 1.114)	-0.280 (-0.459 to -0.137)	0.9944	Acceptable
ALP	1.000 (0.966 to 1.016)	3.000 (2.040 to 5.000)	0.9977	Acceptable
ALT	0.996 (0.982 to 1.004)	0.105 (-0.186 to 0.549)	0.9995	Acceptable
AMY	1.000 (1.000 to 1.000)	0.0 (0.0 to 0.0)	0.9998	Acceptable
AST	1.058 (1.010 to 1.120)	-0.878 (-2.116 to 0.235)	0.9960	Acceptable
DBIL	1.000 (1.000 to 1.034)	0.000(-0.005 to 0.000)	0.9993	Acceptable
TBIL	0.991 (0.966 to 1.000)	-0.016 (-0.020 to 0.000)	0.9993	Acceptable
CA 125	1.027 (1.016 to 1.038)	-0.2656 (-0.8344 to 0.1033)	1.0000	Acceptable
CA 19-9	1.005 (0.955 to 1.034)	-0.3446 (-0.9598 to 0.4621)	0.9979	Acceptable
CA	0.989 (0.937 to 1.036)	0.079 (-0.331 to 0.542)	0.9916	Acceptable
CEA	0.987 (0.963 to 1.019)	0.0572 (-0.0669 to 0.1761)	0.9992	Acceptable
CHOL	1.033 (1.011 to 1.052)	-4.294 (-7.763 to 0.344)	0.9983	Acceptable
CK	1.000 (0.994 to 1.002)	0.0 (-0.3 to 0.7)	0.9999	Acceptable
CL	0.981 (0.952 to 1.000)	1.98 (0.00 to 4.98)	0.9950	Acceptable
CO2	1.000 (0.933 to 1.040)	-0.05 (-0.80 to 1.20)	0.9816	Acceptable
CREA	1.000 (0.996 to 1.000)	0.000 (0.000 to 0.005)	1.0000	Acceptable
hs-CRP	1.000 (0.996 to 1.007)	0.004 (-0.114 to 0.081)	0.9987	Acceptable
CRP	1.000 (0.994 to 1.007)	0.045 (-0.088 to 0.220)	0.9992	Acceptable
LDL	1.009 (0.993 to 1.026)	-0.489 (-2.238 to 1.224)	0.9988	Acceptable
FER	1.032 (0.985 to 1.048)	-1.1126 (-12.9257 to 12.5014)	0.9987	Acceptable
FPSA	1.012 (0.988 to 1.032)	-0.0005 (-0.0042 to 0.0023)	0.9993	Acceptable
GGT	1.000 (0.988 to 1.000)	0.0 (0.0 to 1.2)	0.9997	Acceptable
GLU	1.011 (0.993 to 1.027)	-1.44 (-2.97 to 0.23)	0.9988	Acceptable
HEM	1.000 (0.750 to 1.214)	-1.500 (-2.964 to 0.000)	0.9505	Not interpret
ICT	1.000 (1.000 to 1.000)	0.00 (0.00 to 0.00)	0.9541	Not interpret
TNI	0.993 (0.970 to 1.015)	-0.619 (-1.224 to -0.095)	0.9991	Acceptable
IRON	1.000 (0.990 to 1.010)	0.000 (-0.110 to 0.140)	0.9992	Acceptable
K	1.032 (1.000 to 1.064)	-0.137 (-0.270 to 0.000)	0.9980	Acceptable
LDH	1.012 (0.917 to 1.111)	14.8 (-21.0 to 56.9)	0.9838	Acceptable
LIP	1.000 (1.000 to 1.056)	1.000 (-0.083 to 1.000)	0.9931	Not interpret
LP	1.003 (0.994 to 1.012)	-0.1131 (-0.4541 to 0.2308)	0.9998	Acceptable
NA	1.018 (0.967 to 1.069)	-2.47 (-9.65 to 4.62)	0.9899	Acceptable
NT-proBNP	0.996 (0.978 to 1.011)	-2.128 (-15.960 to 16.839)	0.9985	Acceptable

PHOS	1.061 (1.000 to 1.103)	-0.235 (-0.366 to -0.030)	0.9907	Acceptable
TPSA	1.001 (0.985 to 1.024)	-0.00152 (-0.02010 to 0.01167)	0.9984	Acceptable
TP	1.104 (1.065 to 1.153)	-0.721 (-1.091 to -0.425)	0.9917	Acceptable
TG	1.006 (0.997 to 1.019)	-1.20 (-2.45 to -0.20)	0.9998	Acceptable
UIBC	1.033 (1.000 to 1.063)	-0.697 (-1.935 to 0.850)	0.9972	Acceptable
HDL	1.066 (1.041 to 1.092)	-2.210 (-3.636 to -0.703)	0.9971	Acceptable
BUN	1.000 (0.979 to 1.012)	0.150 (-0.062 to 0.390)	0.9995	Acceptable
URIC	1.008 (1.000 to 1.014)	-0.050 (-0.087 to -0.010)	0.9998	Acceptable

For the slope and intercept, the 95% confidence interval (95% CI) is indicated with parentheses. Results cannot be interpreted because most measured values are below the lowest detection limit of the assay.

Discussion

Various blood-collection tubes are available on the market for efficient laboratory testing as well as for storing blood samples for an appropriate period. Each tube type is designed for using samples of different types and quantities with different anticoagulant techniques. Each manufacturer typically provides recommendations for the centrifugation of serum or plasma at different speeds and times, which are followed by most laboratories. According to the Clinical & Laboratory Standards Institute (CLSI) guidelines, the centrifugation time and g-force recommended by the manufacturer of blood collection tubes [10] or those by the WHO should be followed. Although nowadays laboratories tend to use fully automated systems, these systems are found to delay the pre-analysis steps. The entire process of centrifugation, starting with queuing, loading, balancing, centrifugation, slowing down to stop, and sample unloading from the centrifuge, depending on the setup and workload, can take a minimum of 15–20 min. Earlier studies have investigated the effects of changing the speed or reducing the time required to ensure a continuous analysis, while maintaining the quality of the analysis [11–14].

Centrifugation for 7 min at 2700×g did not affect the chemical and immunological assays in both vacutainer and vacuette tubes. Although statistically significant differences were observed, the percentage of differences was within the acceptable TEa limit. The number of tests performed exceeded the acceptable limit (<5%), which is consistent with the results reported by Minder et al. [4]. No significant differences were observed when whole blood samples were centrifuged as per the WHO guidelines (15 min at 2180×g, 10 min at 2180×g, and 7 min at 1870×g) to reduce the TAT significantly. Additionally, Tanitsaranon et al. [11] reported that centrifugation can be carried out at 1300×g for 10 min. Further, the results obtained when centrifugation was carried out for 7 min at 2200×g and 5 min at 2750×g using lithium heparin vacutainer tubes were found to be acceptable. These results suggest that centrifugation should be performed at higher speeds for shorter times to improve the TAT.

Chemical and immunological tests were performed by centrifugation at 2700×g for 5 min using both vacutainer and vacuette tube types. Statistically significant differences were observed, although the percentage difference was within the acceptable TEa limit. Except for the LDH test that was carried out in both vacutainer and vacuette tubes, only six samples, or 15% and 8 samples or 20% of the total number of cases, respectively, were found to have a percentage difference outside the TEa limit. This finding is in contrast to that reported by Koenders et al. [12], who did not find any differences between serum and plasma samples when the centrifugation duration was reduced from 10 min to 5 min at 1885×g. However, similar results were reported by Moller et al. [13], who found that when the serum and plasma samples were centrifuged for 10 min at 2200×g and 5 min at 3000×g, not only did the overall LDH test results exceed the total error acceptance limit; however, the were higher after centrifugation for 5 min at 3000×g, even in a vacutainer tube, owing to the higher g-force (2700×g).

In a study conducted by Cadamuro et al. [14], results of centrifugation performed on samples of serum and plasma vacutainer tubes for 10 min at 2000×g and 7 and 5 min at 3000×g demonstrated that the serum and heparin samples could be centrifuged at higher speeds (3000×g) for a shorter duration (5 min) when using plasma for blood collection. However, a separate LDH reference value may be required for this.

In this study, it was found that centrifugation performed for 7 and 5 min at 2700×g, performed as per the manufacturer's recommendation, did not affect the HEM index. As listed in Table 2, we did not find any increase in the K, PHOS, and AST levels in the plasma. A comparison of the LDH values of the vacutainer and vacuette tubes demonstrated that the vacutainer tubes had higher LDH values. Centrifugation for 5 min at 2700×g in vacuette tubes increased the LDH values, probably because of the shorter centrifugation period, distinct tube characteristics, and a difference in the lithium content from that of vacutainer tubes. Both vacutainer and vacuette tubes use spray-dried lithium heparin. According to the IFCC [15] recommendation for LDH

measurements, serum is the preferred sample for LDH to avoid platelet aggregation or platelet rupture. The increase in LDH levels may have been due to platelet lysis or optical interference caused by intact platelets [16]. This is consistent with the study by Lippi et al. [6], which investigated lithium heparin tubes being centrifuged at 1200×g for 1, 2, 5, 10, and 15 min. They found that centrifugation time was inversely related to the residual blood cell composition measured in the plasma. Plasma platelet counts were significantly increased in samples centrifuged for 10 min or less, while red and white blood cell counts were significantly increased in samples centrifuged for 2 min or less and for 1 min, respectively. While the LDH of vacutainer tubes after centrifugation for 7 and 5 min at 2700×g became lower than that after 10 min at 1300×g, it was found that increasing the g-force resulted in a decrease in LDH values, possibly due to the lower platelet count. This is consistent with the study conducted by Jestling et al. [17], who found that centrifugation for 10 min at 3000×g significantly reduced platelet counts in plasma compared with centrifugation at 2000×g. However, for routine centrifugation, plasma lithium heparin is often preferred. Research by Minder et al. [4] demonstrated a reduction in centrifugation time from 15 min to 7 min, representing a 47% decrease. Similarly, Koenders et al. [12] reported a reduction to 5 minutes, a 66% decrease compared to WHO guidelines, significantly enhancing laboratory turnaround time (TAT). Consistent with these findings, reducing centrifugation time from 10 min to 7 min resulted in a 30% decrease, leading to a substantial reduction in laboratory TAT for both routine and stat samples, by an average of 19% and 24%, respectively, depending on the workload of each cycle.

Conclusion

This study was designed to determine the appropriate g-force and time for centrifugation using different combinations of vacutainer and vacuette tubes to reduce the centrifugation duration and improve the TAT in the laboratory. The resultant efficiency of centrifugation procedure when carried out according to the manufacturer's guidelines and the conditions designed by us, while maintaining the efficiency, effectiveness, quality, accuracy, and reliability of centrifugation, were compared. We found that centrifugation at 2700×g for 7 min could be used for both vacutainers and vacuette lithium heparin tubes. This study can significantly impact the turnaround time, enabling clinicians to make accurate and timely diagnoses, allowing for immediate adjustments to treatment plans, particularly for critically patients. This improvement increases the likelihood of effective treatment, mitigates the risks associated with prolonged test result waiting times, and enhances patient satisfaction. The scope of future research will aim at studying the impact of the varying needs and constraints under which laboratories operate, including workload, personnel, automated analyzers, the use of different brands or types of tubes, and the determination of TAT taking each of these into careful consideration.

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Declaration of conflict of interests

The authors of this article declare that there is no conflict of interest with regard to the content of this manuscript.

Authors's contributions

Pensiri Choosongsang was the principal investigator, conceived, designed, and performed the experiments, performed data curation, and analyzed data and for editing the manuscript. Yupawadee Yamsuwan designed and performed the experiment and editing the manuscript. Sarayut Petchaithong, Thawin Prasongsab and Naphatohn Bhornsrivathanyou collected specimens and performed the experiment. Phattanapong Choosongsang performed data curation and analyzed data, and was responsible for drafting and editing the manuscript. The final version of the article was reviewed and approved by all authors.

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