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Assessment and establishment of a reference interval for Roche Cobas t 711 coagulation analyzer for a hospital in China

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ABSTRACT

Introduction: Due to the use of different detection reagents and methods, coagulation analyzers can produce different results. Therefore, detection instruments, reagents and methods are important factors affecting the results of coagulation test. Therefore, this paper aims to establish reference intervals applicable to our laboratory for the Roche Cobas t 711 for routine coagulation assays.

Methods:We completed a preliminary evaluation of the analytical performance of the cobas t 711 before any experiment. Healthy volunteer recruitment and ostensibly healthy patients via physical examination were performed to collect individual reference samples. Data were grouped and compared according to age, and the Z test was used to determine whether there was a statistically significant difference between the mean values after grouping.

Results: The self-established PT, APTT and TT reference intervals were 8.4–10.2s, 26.8–42.3s and 14.5–17.1s, respectively. The reference ranges of FIB, AT and DD for people aged 50 years or below were 1.85–3.78 (g/l), 83.9–113.2 (%) and 0–0.45 (mg/l), respectively, and those for people older than 50 years were 2.22–3.86 (g/l), 76.0–112.0 (%) and 0–0.52 (mg/l), respectively.

Conclusion: The self-built reference intervals for the Roche t 711 were basically consistent with those in the instructions, except the APTT ranges were slightly wider. Laboratories should establish applicable reference intervals according to their own conditions to provide guidance for the diagnosis, monitoring and prognosis of clinically related diseases.

1. Introduction

Routine coagulation assays widely used and often play an important role in preoperative screening, bleeding risk assessment and anticoagulant drug therapy monitoring. It is particularly important to determine whether the results of coagulation function tests are normal [1,2]. Therefore, the reference interval is an important criterion for judging whether the clinical test results are normal and also forms an important basis for diagnosing diseases and decision-making. At present, the main routine coagulation function tests performed in clinical laboratories are prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT),

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Table 1
Description of analyzers, reagents, and methods used for this study.

Test	Analyzer	Reagent	Method
PT	CS 5100	Siemens Thromborel S	Clotting assay with lyophilized human placental thromboplastin as activator.
APTT	CS 5100	Siemens Dade Actin FSL Activated PTT Reagent	Clotting assay with purified soy phosphatides and rabbit brain phosphatides in 1.0 × 10-4 M ellagic acid as activator.
TT	CS 5100	Siemens Test Thrombin Reagent	Clotting assay with 1.5 IU/mL of bovine thrombin、bovine albumin as activator.
Fib	CS 5100	Siemens Dade Thrombin Reagent	Clauss Clotting assay. Lyophilized bovine thrombin preparation (approx. 100 IU/mL) with stabilizers and buffers.
AT	CS 5100	Siemens Berichrom Antithrombin III (A)	The antithrombin III in the sample is converted by heparin into an immediate inhibitor and inactivates the thrombin present. The residual thrombin content is determined in a kinetic test measuring the increase in absorbance at 405 nm according to the following reaction.
DD	CS 5100	Siemens INNOVANCE D-Dimer	Polystyrene particles covalently coated with a monoclonal antibody (8D3)10 are aggregated when mixed with samples containing D-dimer.
PT	cobas t 711	Cobas PT Rec C	Lyophilized, recombinant human thromboplastin reagent containing a heparin-neutralizing substance, calcium chloride, stabilizers and buffers that initiates the activation of the extrinsic coagulation cascade when added to citrated human plasma.
APTT	cobas t 711	Cobas APTT Screen	Plasma is pre-incubated with the aPTT Screen reagent, containing a mixture of silicon dioxide particles as activator and a blend of purified soy phosphatides with added buffer, stabilizers and preservative.
TT	cobas t 711	Cobas TT	The TT assay is a buffered thrombin reagent containing 2–10 NIH Units/mL bovine thrombin.
Fib	cobas t 711	Cobas Fibrinogen	Clauss Clotting assay. Lyophilized bovine thrombin contains approximately 100 NIH units/mL with stabilizers and buffers.
AT	cobas t 711	Cobas AT	Heparin and a predefined amount of thrombin are added to the sample in excess. All antithrombin present is bound in an inactive complex.
DD	cobas t 711	Cobas D-DI2	Particle-enhanced immunoturbidimetric assay.
PT	TOP 700	HemosIL RecombiPlasTin 2G	Clotting assay with human recombinant thromboplastin as activator.
APTT	TOP 700	HemosIL SynthASil	Clotting assay with colloidal silica as activator.
TT	TOP 700	HemosIL Thrombin Time	Clotting assay with Purified 15 UNIH/vial bovine thrombin as activator.
Fib	TOP 700	HemosIL Fibrinogen-C XL	Clauss Clotting assay. Lyophilized bovine thrombin preparation (approx. 35 UNIH/mL) with stabilizers and buffers.
AT	TOP 700	HemosIL Liquid Antithrombin	The activity of residual FXa in plasma was quantified by hair color substrate method
DD	TOP 700	HemosIL D-Dimer HS	Immunoturbidimetry of polystyrene latex particles coated with F(AB ′)2 fragment was performed using mouse monoclonal antibody (MA-8D3)

fibrinogen (FIB), D-dimer (DD), and antithrombin (AT). Because of the use of different detection reagents and methods, certain differences can arise in the detection results from instruments. The Roche Cobas t 711 is an automatic blood coagulation analyzer recently introduced in China. The analyzer has a capacity of 225 samples and is capable of performing up to 390 tests per hour, as well as automatic checking of sample tube pressure and quality, to address the presence of relevant concentrations of interfering substances such as cell-free hemoglobin, bilirubin, and lipids [3]. The Roche Cobas t 711 can greatly increase the detection speed to meet the clinical needs of mass/emergency tests; It is worth mentioning that the reference range listed in the instruction manual is based on healthy people from Europe-America, but ranges specific to the Chinese population have not yet been reported. Therefore, a reference interval based on this population and suitable for individual laboratories according to their own factors needs to be established.

2. Materials and methods

2.1. Study design and participants

The study was performed between May and June 2021 according to CLSI C28-A3 guidelines [4]. There are two categories of reference individuals, one being a patient cohort from medical examination department, and the other healthy volunteers. We recruited ostensibly healthy patients and healthy volunteers to collect reference individual samples. The blood samples from ostensibly healthy patients screened during a physical examination were anonymized samples that were destined to be discarded after routine blood clotting tests had been performed in the laboratory, so it was not necessary to obtain informed consent from the patients. The blood samples from the healthy volunteers were obtained with their informed, written consent and after they had signed a health commitment letter for confirmation. The experimental results obtained in this study were only used for this analysis and have not been reported elsewhere. After the performance of the Cobas t 711 was verified, the samples from the reference individuals were tested following daily quality control procedures. All assays and instruments were used according to their respective manufacturers' instructions.

2.2. Reagents

Reagents used on t 711 are listed below: PT Rec (Lot: 47493001)、APTT Screen (Lot: 51761101)、CC 25 mM (Lot: 51760801)、Fibrinogen (Lot: 51632701)、Owren B (Lot: 47497901)、TT (Lot: 50375401)、D-DI 2 (Lot: 49814701)、AT (Lot:50763701)、Cal

Plasma (Lot: 52152401)、D-DI2 Cal Set(Lot: 50361701)、Global Cal (Lot: 49578701)、Blank Cal(Lot: 51117301)、TT (Lot: 50375401)、Con 1 (Lot: 52142001)、Con 1 (Lot: 49402401)、Con 2(Lot: 49404101)、Con 4 (Lot: 49405701)、Con N (Lot: 49578301)、Con P (Lot: 48084901)、Con P+ (Lot: 53140401)、D-DI2 Con (Lot: 49284901). The reagents and methods used in the three analyzers are shown in Table 1.

2.3. Sample collection

All samples used in this study were collected through sphenoid needle venipuncture. The first tube of blood which obtained with no additives was discarded, and the second tube of blood obtained in a test tube containing 108 mmol/l sodium citrate anticoagulant. Blood was collected at a strict ratio of 1: 9 anticoagulant to blood. The blood was centrifuged at 2500 g for 15 min and plasma separated stored at −80 °C. The sample set was thawed and immediately put on the machine for testing.

2.4. Inclusion and exclusion criteria

2.4.1. Inclusion criteria

a) Ostensibly healthy patients with normal coagulation results detected by other blood coagulation instruments;
b) Healthy volunteers after they had signed a health commitment letter for confirmation

2.4.2. Exclusion criteria

a) Alcohol abuse;
b) Long-term or recent blood donation and transfusion;
c) Abnormal blood pressure;
d) Symptoms of recent and past diseases, including coagulation disorders and diseases of the blood system, thromboembolic diseases, hemorrhagic diseases, heart and cerebrovascular diseases, malignant tumors, abnormal liver and renal function; or a history of infection, fever or surgery within the last 30 days;
e) Pregnancy, lactation or menstruation;
f) Drugs, including those currently in use that may affect blood clotting and fibrinolysis, including but not limited to acetylsalicylic acid, oral anticoagulants, warfarin, heparin, antiplatelet drugs and contraceptives;
g) Special diets

2.5. Reference interval grouping

Data were grouped and compared according to age, and the Z test was used to determine whether there was statistically significant difference between the mean values after grouping. If there were significant differences between the two groups, at least 120 individuals were included in each group to establish the reference intervals of the different populations. The reference limit was adjusted appropriately to form a reference interval based on clinical significance and practical clinical opinions. For example, the lower limit of DD detection has no clinical significance, and thus only a unilateral reference line was set. Finally, we compared the test results and reference intervals of the Cobas t 711 with those of other systems, such as the TOP 700 and CS 5100.

2.6. Analytical assessment

According to “Analytical quality specifications for routine tests in clinical hematology” (Standard No.: WS/T 406 2012) [5], determine performance validation methods and quality requirements for analysis. Imprecision Studies: Two clinical samples with two concentration levels (including normal and abnormal) were taken, and each sample was tested 11 times in accordance with the conventional method. The arithmetic mean and standard deviation of the test results of the last 10 times were calculated, and the coefficient of variation was calculated to evaluate the within run precision. The normal and abnormal concentrations of quality control products were selected and determined 4 times a day for 5 days. The coefficient of variation was calculated to evaluate the between run precision. Linearity: There is a high concentration plasma (H) close to the upper limit of expectation and a plasma (L) close to the lower limit of expectation, and the dilution is conducted according to the ratio of 4H, 3H+1L, 2H+2L, 1H+3L and 4L, respectively. The detection of each dilution is repeated twice, and the measured value is compared with the theoretical value to verify the linear range. Methods comparison studies: Six coagulation detection results of three coagulation analyzer systems were compared, and linear correlation analysis were conducted.

2.7. Statistical analysis

The reference ranges were calculated based on the distribution of the results for PT, APTT, TT, FIB, DD and AT results. The normality of the data was assessed using the Kolmogorov-Smirnov test. If the data followed a normal distribution, the reference range was calculated using the mean ± 1.96* standard deviation. For non parametric distribution, the median and interquartile spacing were used, and the reference range was determined by the percentile method (using 2.5% and 97.5% percentiles).

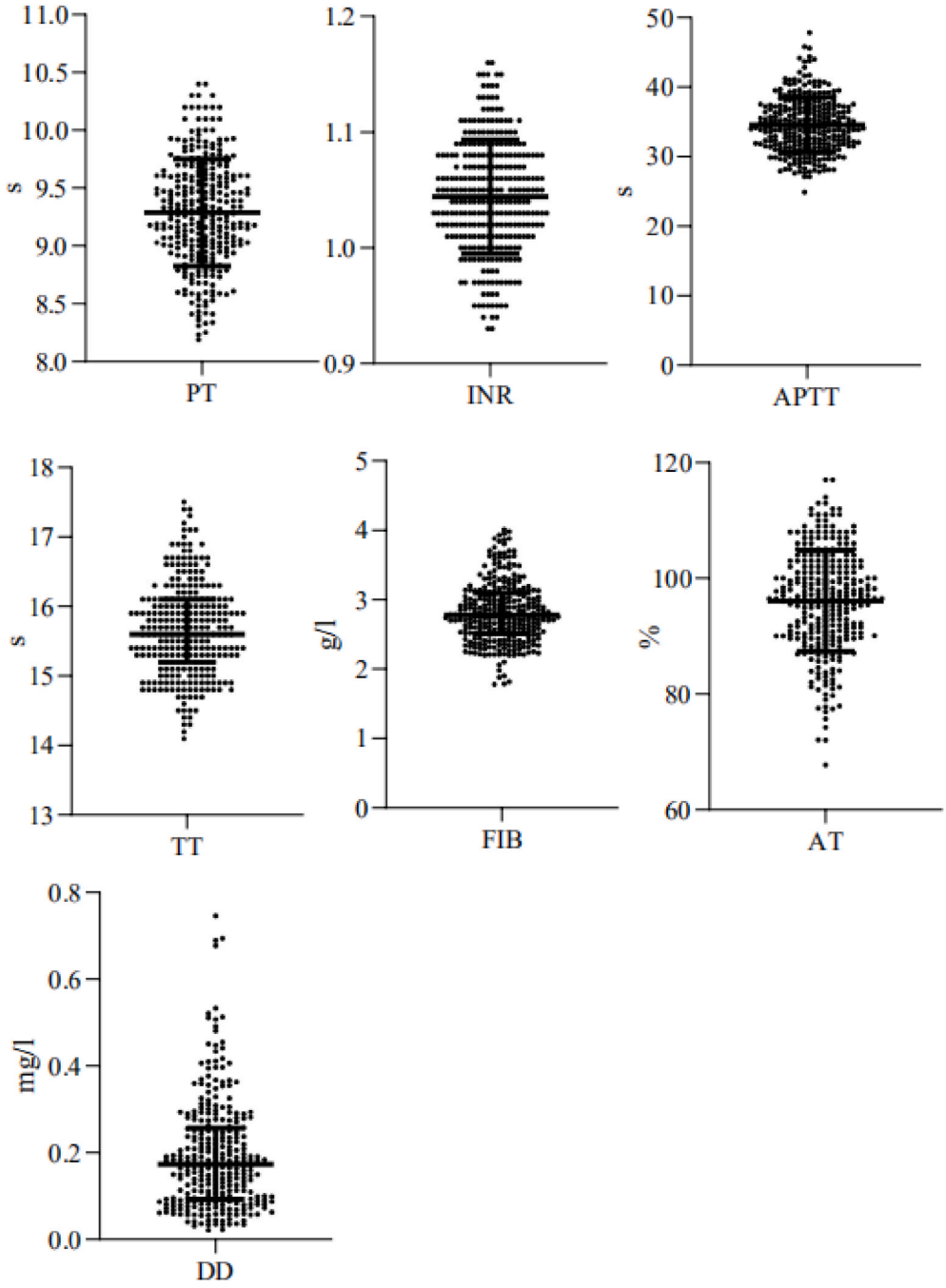

Fig. 1. Distribution of blood coagulation tests for the reference population.

3. Results

3.1. Baseline characteristics

A total of 330 samples were collected, and 297 individuals were included in the final analysis after excluding outliers and abnormal results. Among them, 42 individuals are healthy volunteers including 22 individuals from Roche and 20 from medical staff, and the rest are ostensibly healthy patients. The age ranged from 18 to 88 years, with an average age of 53 (33, 67), and the sex distribution was 145 males and 152 females. The detailed results are shown in Figs. 1–3.

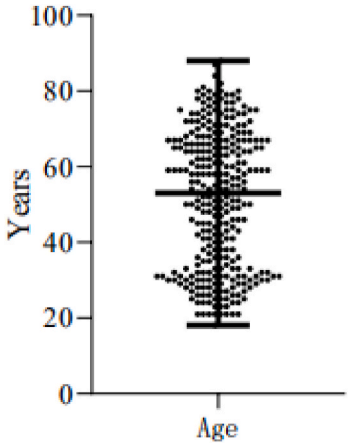

Fig. 2. Age distribution of the reference population.

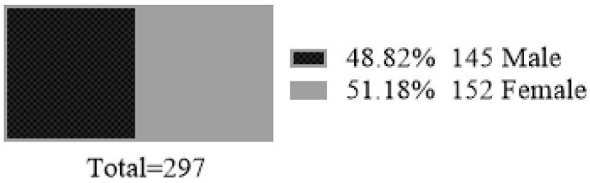

Fig. 3. Sex distribution of reference population.

Table 2
Comparison of blood coagulation tests results between the young group and elderly group.

	Young group (50 years and below)	Elderly group (older than 50 years)	t/Z	P
Volunteers (n)	137	160		
Age (years)	34 ± 8.7	66 ± 8.6		
Male (n)	42	103		
PT (s)	9.3 ± 0.5	9.3 ± 0.4	1.49	0.14
INR	1.05 (1.02,1.09)	1.04 (1.01, 1.07)	−1.66	0.10
APTT (s)	34.3 ± 3.8	34.8 ± 4.1	−0.93	0.35
TT (s)	15.6 (15.1,16.0)	15.7 (15.3, 16.1)	−1.35	0.18
FIB (g/l)	2.65 (2.38, 2.90)	2.89(2.65, 3.17)	5.24	0.01
AT (%)	98.5 ± 7.5	94.0 ± 9.2	4.61	0.01
DD (mg/l)	0.14 (0.08, 0.20)	0.20 (0.13, 0.29)	5.05	0.01

3.2. Reference interval establishment

There were no statistically significant differences in PT, APTT or TT according to age (Young group: 50 years and below; Elderly group: older than 50 years), while there were significant differences in FIB, AT and DD. The self-established PT, APTT and TT reference intervals were 8.4–10.2s, 26.8–42.3s and 14.5–17.1s, respectively. The reference ranges of FIB, AT and DD for people aged 50 years or below were 1.85–3.78 (g/l), 83.9–113.2 (%) and 0–0.45 (mg/l), respectively, and those for people older than 50 years were 2.22–3.86 (g/l), 76.0–112.0 (%) and 0–0.52(mg/l), respectively. The detailed results are shown in Tables 2–3.

3.3. Analytical assessment

3.3.1. Imprecision Studies

The results of imprecision studies are shown in Table 4. Meet the quality analysis requirements of the WS/T 406 2012 and manufacturers.

4. Linearity studies

The linearity studies showed excellent performance as described in the instructions. Fib linear verification ranges from 0.6 to 9 g/l ($y = 1.0003x+0.105$, $R^2 = 0.999$), DD linear verification ranges from 0.20 to 9 mg/l ($y = 0.9871x-0.2257$ $R^2 = 0.9992$), AT linear

Table 3
Self-built reference ranges for the t 711 and comparison with other systems (95% confidence interval).

		PT (s)	INR	APTT (s)	TT (s)	FIB (g/l)		AT (%)		DD (mg/l)	
Reference range	CS 5100[a]	9.8–12.1	/	25.0–31.3	14–21	1.8–3.5		75–125		0–0.55	
	TOP 700[a]	9.4–12.5	/	25.4–38.4	10.3–16.6	2.38–4.98		83–128		0–0.5	
	Cobas t 711[a]	8.4–10.6	/	23.9–33.2	16.1–19.7	1.9–4.1		76.9–114		0–0.5	
Self-built reference ranges for the t 711	Grouped by age (Years)	18–88				18–50	51–88	18–50	51–88	18–50	51–88
		8.4–10.2	1.0–1.2	26.8–42.3	14.5–17.1	1.85–3.80	2.22–3.86	83.9–113	76.0–112	0–0.45	0–0.52
	Ungrouped					2.08–3.85		79.0–113		0–0.51	

[a] As quoted by manufacturers.

Table 4
Results of imprecision studies on Cobas t 711 analyzer.

Test	Within run (n = 20)		Between run (n = 40)	
	Values(Mean±SD)	CV%	Values(Mean±SD)	CV%
PT(S)				
Normal	9.1 ± 0.1	0.4	9.2 ± 0.1	0.9
Abnormal	26.5 ± 0.2	0.7	27.2 ± 0.5	1.7
APTT(S)				
Normal	32.5 ± 0.2	0.7	31.7 ± 0.3	0.9
Abnormal	58.4 ± 0.2	0.3	58.6 ± 0.6	0.9
TT(s)				
Normal	16.9 ± 0.2	1.3	16.8 ± 0.2	1.3
Abnormal	40.8 ± 0.4	0.9	40.4 ± 1.1	2.7
Fib (g/l)				
Normal	2.84 ± 0.11	3.87	2.65 ± 0.08	2.97
Abnormal	1.35 ± 0.03	2.06	1.28 ± 0.03	2.51
AT(%)				
Normal	96.6 ± 1.5	1.6	92.8 ± 3.6	3.9
Abnormal	37.7 ± 0.7	1.9	35.5 ± 2.5	7.1
DD (mg/l)				
Normal	0.92 ± 0.01	0.60	0.90 ± 0.04	4.24
Abnormal	3.83 ± 0.01	0.28	3.64 ± 0.13	3.64

verification ranges from 15.0 to 150% ($y = 0.9973x-0.4727$, $R^2 = 0.9994$).

5. Methods comparison studies

We compared the reference intervals and twenty test results from the Cobas t 711 with the results from the TOP 700 and CS 5100. The results of Cobas t 711 showed a linear relationship with those of the other systems, except that the APTT was slightly different. The results of methods comparison are shown in Figs. 4–5.

6. Discussion

The assessment for Roche Cobas t 711 meet the requirements of clinical testing. The self–built reference intervals on the Roche coagulation analyzer were basically consistent with those of the instructions and showed a linear relationship with those of other systems, the TOP 700 and CS 5100, except that the APTT range was slightly wider. Coagulation analyzer systems have different reference ranges, and laboratories need to establish their own reference ranges according to their individual conditions.

Clotting tests play an important role in many fields, including the recent outbreak of COVID-19. Therefore, rapid and accurate detection of coagulation function is conducive to the clinical judgment of the patient's condition, diagnosis and prognosis. Coagulation function tests are also used for disseminated intravascular coagulation (DIC) screening and cardiovascular disease diagnosis and are important indicators for monitoring various anticoagulant drugs. Furthermore, they are particularly important for laboratory technician in reporting accurate clinical results. Consequently, the establishment of a personalized reference interval is conducive to accurately judging the degree to which a test value is abnormal. Providing patient-specific treatment monitoring will greatly aid in the development of individualized, precision medicine. The high reagent loading capacity coupled with the automatic reagent reconstitution of the Cobas t 711 analyzer will contribute to shorter turnaround times (TAT) and improve laboratory efficiency [6].

Studies have shown that DD and FIB are higher in elderly individuals, which may be associated with endothelial damage. FIB is not only a coagulation factor but also an inflammatory marker, which may be elevated in elderly individuals due to vascular sclerosis or the presence of multiple infections. APTT is affected by many factors, such as endogenous coagulation factors, regional, population characteristics, and blood collection [7]. This may be one reason why our APTT reference interval is wider than that in the manual. Another reason for this wider interval may be the difference reagent composition and content [8]. On the other hand, the proportion of healthy volunteers is relatively small, whichh may leads to the detection deviation of reference individuals. In addition, we found that the PT of Roche t711 is smaller than that of TOP 700 and CS 5100. Therefore, the INR value should be paid attention to when evaluating the monitoring of oral warfarin treatment. In conclusion, when the laboratory has two or more sets of blood coagulation detection systems, it is recommended to use the same system for testing the same patient to facilitate monitoring.

In this study, we were able to confidently establish reference intervals based on the performance verification for the Cobas t 711. Some other reports have described the performance of the Cobas t 711 in detail [9] [–] [10]. We divided into groups based on the age of 50 and established a reference interval to reduce the impact of individual related variables on routine coagulation parameters in Chinese healthy population [11]. However, This study is a single center study. The inclusion of multi center and large sample reference individuals will be more conducive to the establishment of reference interval of Roche automated coagulation analyzer in Chinese population. In addition, there are also differences in the blood coagulation test results between people from different regions, which can also lead to different ranges when establishing the reference intervals. Furthermore, different manufacturers or types of detection reagents from the same manufacturer can have different sensitivities to the reaction, which can also lead to inconsistent detection

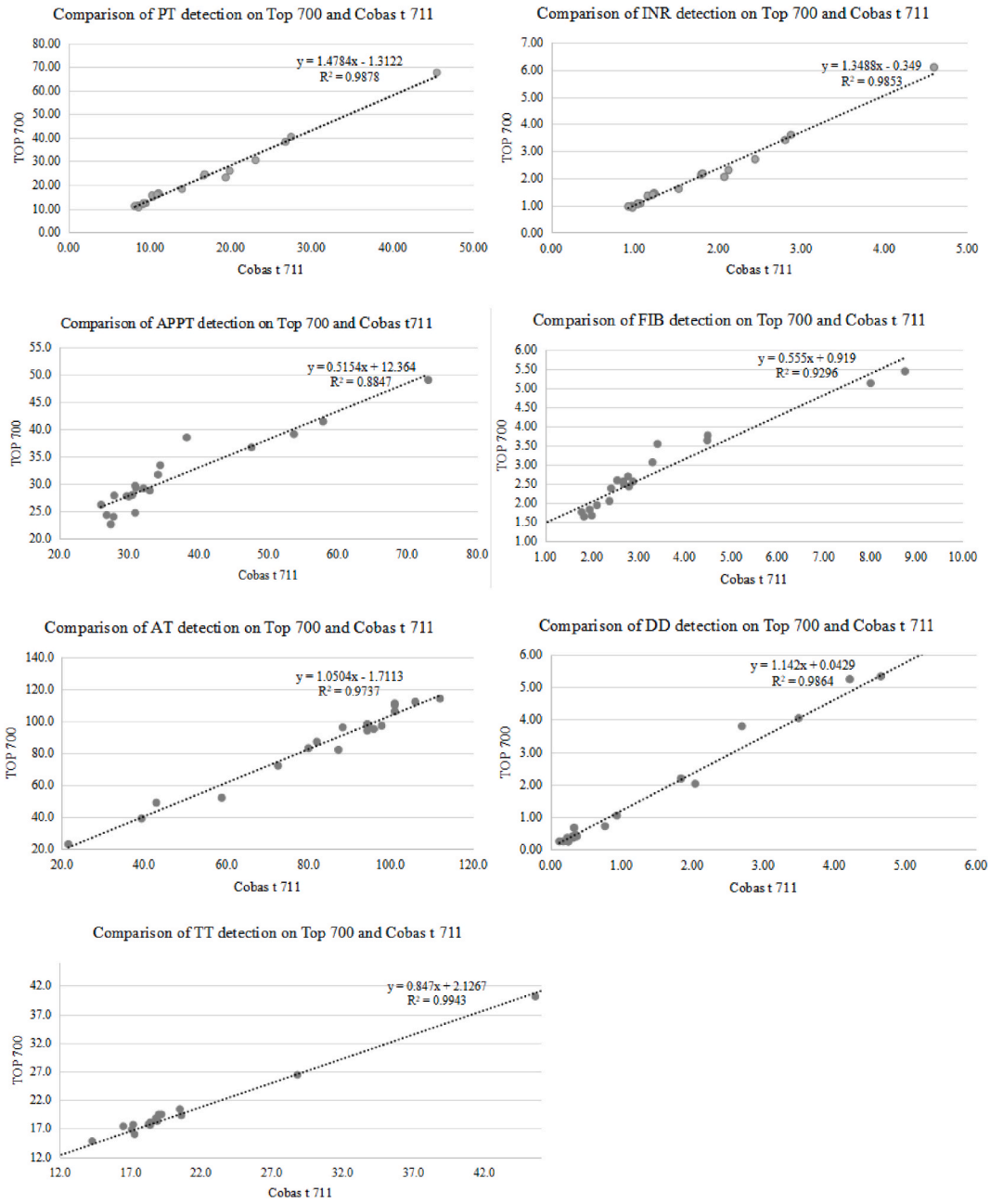

Fig. 4. Comparison of blood coagulation test results between the Cobas t 711 and TOP 700.

results.Thus, our study only aimed to establish individualized reference intervals for our hospital and its currently used reagents. The reference ranges for different laboratories may vary as a result of sampling from a different population of healthy individuals and the use of different technologies, methods, instruments and experimental operations; therefore, the reference range established in this study may provide a guide for other laboratories, but that these laboratories will still be required to verify local ranges.

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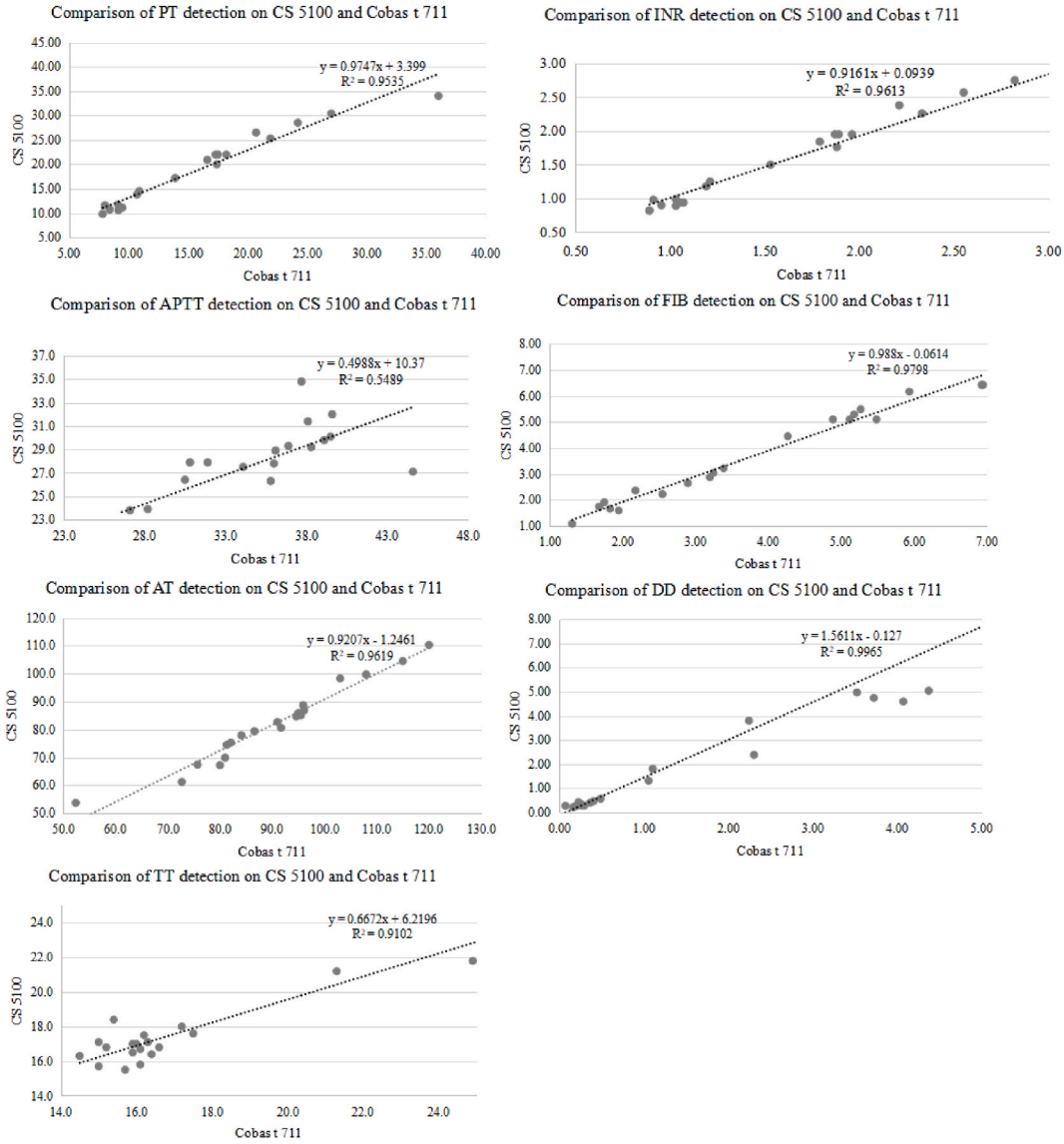

Fig. 5. Comparison of blood coagulation test results between the Cobas t 711 and CS 5100.

Informed consent

Informed consent was obtained from all individuals included in this study.

Authors' contributions

All authors have accepted responsibility for the entire content of this manuscript and have approved its submission.

Declaration of competing interest

The authors state no conflicts of interest.

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References

[1] Q. Ji, Q. Xu, Z. Wang, et al., Association between activated partial thromboplastin time, age and bleeding evets in NVAF patients receiving dabigatran [J], Eur. J. Clin. Pharmacol. 75 (3) (2019) 321–328.

[2] M.F. Ruberto, F. Marongiu, Are prothrombin time and clot waveform analysis useful in detecting a bleeding risk in liver cirrhosis [J], Int J Lab Hematol 41 (1) (2019) 118–123.

[3] G. Lippi, G. Salvagno, M. Gelati, G. Poli, D. Giavarina, E. Favaloro, Analytical assessment of the new Roche cobas t 711 fully automated coagulation analyzer, Semin. Thromb. Hemost. 45 (2019) 308–314.

[4] Clinical and Laboratory Standards Institute (CLSI), Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline—Third Edition. CLSI Document C28-A3, Clinical and Laboratory Standards Institute, Wayne, PA, 2008.

[5] National Health and Family Planning Commission of the P.R.C, Analytical Quality Specifications for Routine Tests in Clinical Hematology[s, Standards Press of China, Beijing, 2013.

[6] Steve Kitchen, Moniek de Maat, Michael Nagler, et al., System performance evaluation of the cobas t 711 and cobas t 511 coagulation analyzers in routine laboratory settings, Blood Coagul. Fibrinolysis 31 (2020) 459–467.

[7] Emmanuel J. Favaloro, Geoffrey Kershaw, Soma Mohammed, How to optimize activated partial thromboplastin time (APTT) testing: solutions to establishing and verifying normal reference intervals and assessing APTT reagents for sensitivity to heparin, lupus anticoagulant, and clotting factors, Semin. Thromb. Hemost. 45 (1) (2019) 22–35.

[8] S. Kitchen, U. Geisen, J. Kappelmayer, et al., Evaluating the analytical performance of five new coagulation assays for the measurement of prothrombin time and activated thromboplastin time, Int J Lab Hematol 40 (2018) 645–654.

[9] G.L. Salvagno, G. Lippi, M. Gelati, G. Poli, E.J. Favaloro, Analytical performance of the new D-dimer and antithrombin assay on Roche cobas t 711 analyzer, Int J Lab Hematol 41 (2019) e54–e56.

[10] S. Kitchen, U. Geisen, J.G. Boehm, J. Kappelmayer, P. Quehenberger, A. Lowe, et al., Evaluating the analytical performance of four new coagulation assays for the measurement of fifibrinogen, D-dimer and thrombin time, Int J Lab Hematol 40 (2018) 637–644.

[11] Jianxin Li, Yanqing Huang, Liping Nie, et al., Effects of individual related variables on routine coagulation parameters in Chinese healthy population, Diagn Theory Pract. (1) (2010).