Improving equivalence in fibrinogen evaluation between the prothrombin time-derived fibrinogen assay and Clauss method using a pooled plasma calibrator

Sheng Wang ,[1] Xiaoyong Duan,[2] Xingtai Liu,[1] Liuchun Wu,[3] Yan Zhang,[1] Xin Jin[1]

[1]Department of Clinical Laboratory, Three Gorges University Third Clinical Medical College, Sinopharm Gezhouba Central Hospital, Yichang, China
[2]Department of Emergency Medical, Three Gorges University Third Clinical Medical College, Sinopharm Gezhouba Central Hospital, Yichang, China
[3]Department of Medical Business, Three Gorges University Third Clinical Medical College, Sinopharm Gezhouba Central Hospital, Yichang, China

Correspondence to
Dr Sheng Wang, Department of Clinical Laboratory, Three Gorges University Third Clinical Medical College, Sinopharm Gezhouba Central Hospital, Yichang 443002, China; 11423144@qq.com

Received 14 March 2020
Revised 16 May 2020
Accepted 26 May 2020
Published Online First 1 July 2020

To cite: Wang S, Duan X, Liu X, *et al*. *J Clin Pathol* 2021;**74**:58–63.

ABSTRACT

Aims Both the Clauss and prothrombin time-derived fibrinogen (PT-Fib) methods have widely been used in fibrinogen (Fib) evaluation. We aimed to improve the interchangeability between these two methods.

Methods Thirty fresh plasmas of low, normal and high Fib concentration were mixed to prepare the pooled human plasma. The Fib concentration was assessed by the Clauss method. The PT-Fib values were further recalibrated by the assigned plasma pools and the precision, linearity and reference intervals were verified according to the guidelines of American Society for Clinical and Laboratory Standards (CLSI) EP15-A and EP6-A documents. Finally, the recalibrated PT-Fib method was compared with Clauss method by the simultaneous Fib test of total 5259 coagulation samples.

Results The results indicated that the recalibrated PT-Fib method can detect the Fib concentration with clinically acceptable third-order linearity in the range of 1.27–8.00 g/L. Only one result out of 39 healthy people tested using the recalibrated PT-Fib method did not fall within the reference range defined by the manufacturer. We also found more than 99% of results between these two methods were interchangeable in the range of 1.51–8.00 g/L. The disagreement between these two methods was found only in patients with certain underlying conditions.

Conclusions After recalibration, the consistency between the PT-Fib and Clauss methods was enhanced and the interchangeability was improved. The application of the recalibrated PT-Fib method provided accurate and reliable results with the manufacturer-provided reagents and improved detection speed and cost-effectiveness.

INTRODUCTION

Fibrinogen (Fib), the precursor of fibrin, is a liver-synthesised blood coagulation glycoprotein.[1] Fib plays a significant role as a thrombin substrate and a fibrinolytic target in both coagulation and fibrinolysis processes and is increasingly attracting clinical attention for its utility in disease detection.[2 3] Decreased concentrations of Fib are correlated with bleeding tendency or an unexplained prolongation of the activated partial thromboplastin time (APTT) or prothrombin time (PT). Elevated levels of Fib may also be associated with enhanced danger of thrombosis.[4–8]

Quantitation of fibrinogen in plasma is an important screening tool for coagulation disorders. Currently, although there are a number of assays to measure fibrinogen levels in plasma, in practice most laboratories use the Clauss method,[9] a functional assay based on the principle that the clotting time is positively correlated with the plasma fibrinogen content. Briefly, after incubation of the diluted plasma at 37°C with calcium followed by phospholipid and thrombin, the time taken to create the clot is likened to a calibration curve and the concentration of fibrinogen is deduced.[10] With excellent specificity, the Clauss method became a reference technique recommended by the WHO,[11] as well as a routine measurement method suggested by National Clinical Testing Standards Committee of the USA.[12] However, the detection speed is slow and the reagents are expensive.

The prothrombin time-derived fibrinogen (PT-Fib) assay is an indirect fibrinogen assay based on changes in optical density during PT determination and the concentrations of fibrinogen are derived from a calibration curve.[10] This detection process requires only PT but not the fibrinogen measurement reagent, which makes it relatively easier to conduct and is cheaper than the Clauss method. In addition, PT reagents are more stable in the reagents compartment compared with Clauss reagents (reagent instructions is 15 days and FIB is 8 hours). It is generally considered that within the normal range, the results derived from the PT-Fib method are close to the results of the Clauss method.[13] The original Werfen manufacture guide states that in the absence of abnormal fibrinogen, the PT-Fib assay can substitute for the Clauss method at Fib normal range of 2.00–4.00 g/L.

However, the Fib gene is polymorphic and causes the products to be highly heterogeneous[14] and its detection is affected by many factors, which can result in disparate findings between instruments and methods. Previous studies revealed that fibrinogen levels measured using these two methods were poorly correlated in patients with some illnesses, regardless of what reagents or instruments were used.[15 16] In addition, in a multicentre study, Mackie *et al* found that the bias between the PT-Fib and Clauss methods is calibrator dependent and the degree of disparity is linked to calibrator and plasma sample turbidity.[17] Based on our prior experience in validation of the manufacturer's protocol, we also found that most of the Fib results were 10% –20% higher according to the PT-Fib method when

Table 1 Comparison between Clauss PT-Fib method in another ACL TOP300 instrument before recalibration

PT-Fib results Range (g/L)	Sample numbers	Results $\bar{x}\pm s$ (g/L)		Bias $\bar{x}\pm s$ (%)	Accordance rate (%)
		PT-Fib	Clauss		
0.77–2.00	32	1.56±0.36	1.42±0.40	11.89±21.72	71.88
2.01–4.00	439	3.20±0.49	2.88±0.48	11.89±11.18	84.97
4.01–11.45	433	5.32±1.25	4.42±0.96	20.37±10.33	51.50

PT-Fib, prothrombin time-derived fibrinogen.

compared with the Clauss method (table 1, figure 1A,B). We suspect that the bias between these two methods is due to using the same calibrator with different value assignments.

In this study, to improve the interchangeability between these two methods, we present a practical protocol to recalibrate the PT-Fib using the pooled plasma value assigned by the Clauss method. The performance and clinical applications of this recalibrated assay have been validated in our laboratory.

MATERIALS AND METHODS

Patients and samples

All samples were collected from both hospitalised patients and outpatients in the Sinopharm Gezhouba Central Hospital. A total of 5259 samples were collected from 3786 patients, including 1955 males and 1831 females ranging from 2 to 95 years of age. These patients were enrolled from November 2018 to April 2019 and the total sample number from different patients was 3786. All data were fully anonymised prior to access and use. This study was approved by the Hospital Ethics Committee with the ethics board number 20181003, and a general consent form was obtained from all patients. For sampling, 1.8 mL of venous blood was placed in a vacuum blood vessel(Wuhan Zhiyuan, Wuhan, China) followed by horizontal centrifuge at 2000×g for 15 min. Samples from patients with haemolysis and lipaemic were excluded (seven with haemolysis, nine with lipaemic). In addition, if hematocrit (HCT) was >55% or <25%, the volume of anticoagulant was recalculated according to the correction formula: (mL)=1.85 ×0.001×blood vol × (1 – HCT).[18]

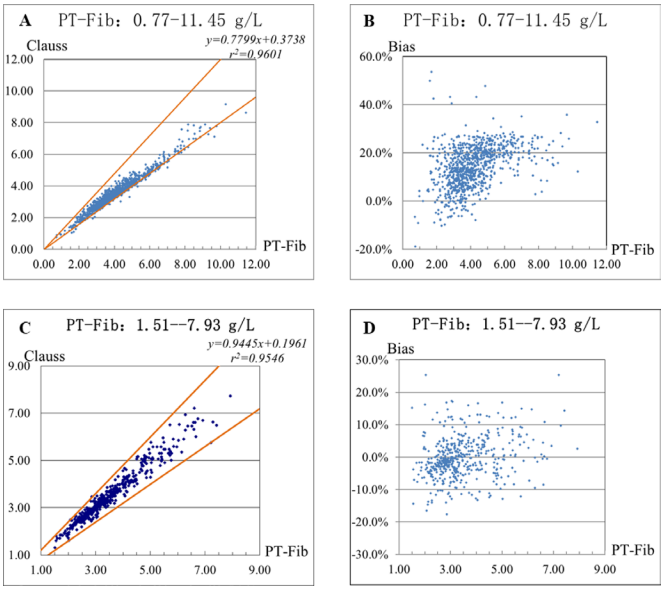

Figure 1 The accordance rates between two methods after recalibration in another TOP300. In the interval of 1.51–7.93 g/L (C,D), 501 of the 503 results met the requirements for consistency (bias ≤20%, between the two plotted lines) and accordance rate was 99.60%. In contrast, the bias was over 20% in the ranges between 0.77 and 11.45 g/L before recalibration (A,B). PT-Fib, prothrombin time-derived fibrinogen.

Freshly pooled human plasma for PT-Fib calibration

Freshly pooled human plasma used for value transfer were prepared from equal volumes of 30 Clauss method-tested fresh specimens with good optical quality. These samples included 4 low-value samples, 23 normal-value specimens and 3 high-value specimens according to the value distribution from 49 753 (low: normal: high, 6751:38207:4795) Clauss tests over the past 2 years. The pooled plasma was tested by the Clauss assay 20 times using an ACL TOP700 automatic coagulation analyzer, with the original reagent (batch number N0688281), calibrator (E0177755) and quality control products (N0386250 and H0864436) all provided by the original manufacturer.[19] The mean values were taken as the assignment value for calibrating the PT-Fib method using an ACL TOP700 with reagent N0386789 and quality control products N0386250 &H0864436 after outliers (greater than three SD from the mean) were excluded. The calibrators were traceable to the National Institute of Biological Products (NIBSC) standard system.

Precision analysis

Per the recommended method described in CLSI's EP-15A document,[20] commercial quality control (QC) reference preparations at both normal and abnormal levels were tested by the recalibrated PT-Fib assay. In particular, for refrigerator storage, QC sample of each level was divided into five aliquots. The fresh aliquot of normal and abnormal commercial QC reference level was prewarmed to room temperature and tested three times a day for five successive days. The results were statistically processed to calculate intra-assay SD (S_r), interassay variance (S_b^2), within-laboratory SD (S_l), degree of freedom (T) and verification value (V). If $S_l<V$, the precision of the manufacturer's claim was verified.

Linearity

Per the CLSI EP6-A document,[21] leftover samples with low (L) and high (H) Fib concentrations were mixed at the volume ratios of 4:0, 3:1, 2:2, 1:3 and 0:4 to obtain five linear target concentrations. The expected value of each sample concentration was calculated according to the formula (CL×VL+CH×VL)/(VL+VH) (where C is the sample concentration and V is the sample volume). Triplicate measurements were carried out in the above linear samples and the mean values were calculated. The measured average value and the expected value were checked to remove outliers, followed by a multiple linear regression analysis and the regression of log-transformed concentration was drawn for assessment.

Table 2 The precision analysis of recalibrated PT-Fib method

Level	Level 2					Level 3				
Replicates	1	2	3	4	5	1	2	3	4	5
Means (g/L)	3.05	3.04	2.98	3.07	3.06	1.95	2.02	1.98	2.09	2.05
S_r	0.085	0.091	0.050	0.071	0.090	0.067	0.047	0.050	0.036	0.031
S_b^2	0.035					0.051				
S_l	0.073					0.064				
T	13.789					8.812				
V	0.100					0.106				

PT-Fib, prothrombin time-derived fibrinogen.

Reference interval

Per the CLSI C28-A2 document,[22] 39 apparently healthy medical examiners were selected for the recalibrated PT-Fib test mentioned above. These individuals were determined to have no underlying disease after a comprehensive medical examination at our hospital. If more than 90% of the Fib values obtained from this method fall within the reference interval specified by the manufacturer, this implies that manufacturer's claim was verified in their calibrated PT-Fib test.

Methods comparison

To investigate how the freshly pooled plasma calibrator had influenced the concordance between the Clauss and PT-Fib assays, 5215 freshly collected samples (within 2 hours) were analysed in parallel by these two methods on an ACL TOP700 and the PT-Fib assay was calibrated by the plasma pools calibrator. The quality control experiments at normal and abnormal levels were conducted daily and verified according to the quality control regulations of the two level sigma. Using the Clauss test as a reference, the proportions of our recalibrated PT-Fib method results that achieved desirable performance requirement per the People's Republic of China Health Industry Standard WS/T 406–2012 (bias no more than 20%) were used to calculate the concordance rates between these two methods across measurement ranges.

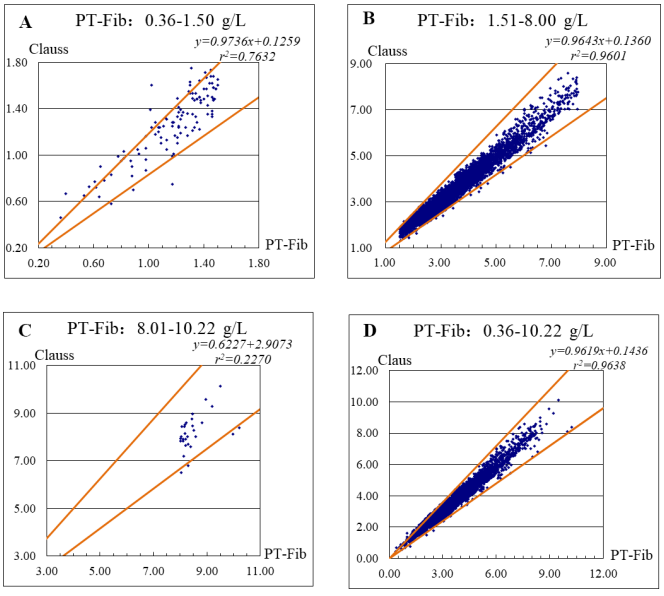

Figure 2 The accordance rates between two methods after recalibration. Using the Clauss method as a reference, concordance between the Clauss and recalibrated PT-Fib methods across Fib measurement ranges was demonstrated. In the interchangeable interval of 1.51–8.00 g/L (B), the determination coefficient r^2 from linear regression was 0.9601, indicating that an excellent agreement between these two methods has been established within this interchangeable interval. In contrast, the determination coefficient r^2 in the other two ranges (A,C) were much lower. (D) The accordance rates between two methods in the interval of 0.36- 10.22 g/L which contains 5215 samples. PT-Fib, prothrombin time-derived fibrinogen.

Statistical analysis

Per the CLSI document EP15-A2, Excel 2010 was used to analyse test information. Continuous variables with normal distribution were presented as the means±SD and compared by Student's t-test. Linear correlation coefficients were tested for significance using analysis of variance. Outliers were defined as values exceeding three SD from the mean of each group. P values less than 0.05 were considered significant.

RESULTS

Performance verification of PT-Fib method after recalibration

The results of the precision verification of the recalibrated PT-Fib method are detailed in table 2. Within-laboratory SD (S_l) (0.073 and 0.064, respectively) was less than verification value (V) (0.1 and 0.106, respectively) at both normal and abnormal commercial QC reference levels, indicating that the precision claimed by the manufacturer was verified. For linearity analysis, there was no obvious bias between the expected Fib value and the measured value derived from the recalibrated PT-Fib method. The best fitting equation was a cubic polynomial with the following regression: $y=-0.0067x^3+0.1143x^2+0.4506x+0.5341$ ($r^2=0.9999$). The imprecision across the five gradient concentrations ranged from −0.44% to 0.45% and the average imprecision was −0.02%, which is much less than the criterion in the WS/T 406–2012 standard (≤10%).[23] These results indicated that our recalibrated PT-Fib method can detect Fib with a clinically acceptable third-order linearity in the range of 1.27–8.00 g/L. Our recalibrated PT-Fib method has also been used to test plasma Fib in 39 healthy people including 22 males and 17 females aged 23–71. The collected results ranged from 2.49 to 5.01 g/L and only one test result did not fall within the reference range of 2.38–4.98 g/L provided by the manufacturer, leading to the agreement rate of 97.44%.

Method comparison

Based on our experience, the disagreement between PT-Fib and Clauss methods was found most often in patients with certain underlying conditions (44 in total, 0.84% of the total sample), including the history of extensive anticoagulant therapy after dialysis, diagnosis of liver sclerosis decompensation, primary or metastasis hepatic cancer, sepsis and myeloma, the level of

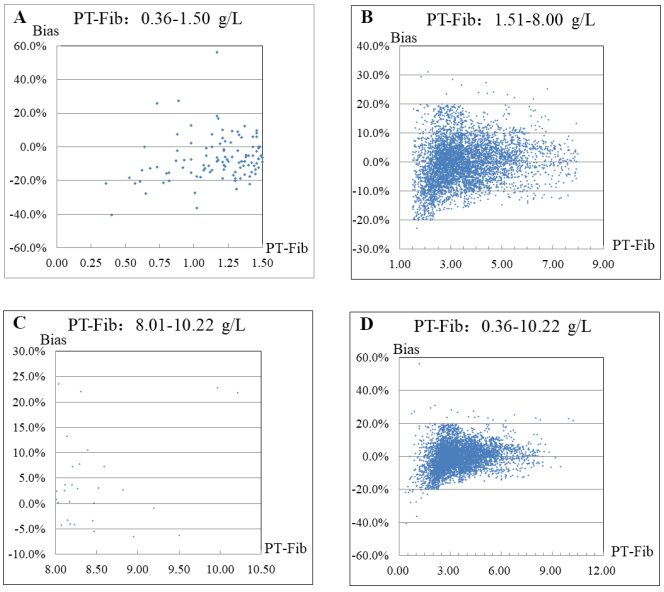

Figure 3 In the interchangeable interval of 1.51–8.00 g/L (B), 5060 of the 5075 results met the requirements for consistency (bias≤20%, between the two plotted lines) and accordance rate was 99.70%. In contrast, the bias was over 20% in the other two ranges (A,C). (D) The bias between two methods in the interval of 0.36- 10.22 g/L which contains 5215 samples. PT-Fib, prothrombin time-derived fibrinogen.

fibrinogen degradation products (FDP) ≥50 mg/L, thrombin time (TT) ≥25 s and APTT≥80 s. In patients without the above underlying conditions, a comparative analysis of these two methods is shown in figures 2 and 3 and summarised in table 3. Using the Clauss method results as the target value, the concordance was defined as the proportion of the results in which bias between the PT-Fib method and Clauss method was less than 20%. As shown in table 3, the accordance rates in the range of 1.51–2.37 g/L, 2.38–4.98 g/L and 4.99–8.00 g/L were all greater than 99%. With the interchangeable interval between the PT-Fib method and the Clauss method set at 1.51–8.00 g/L, the results of 5075 samples fell within this range. Further, 5060 of the 5075 samples met the requirements of consistency (bias≤20%) and the accordance rate was 99.70%. As shown in figure 2B, the determination coefficient r^2 from linear regression was 0.9601, indicating an excellent agreement between these two methods has been established within this interchangeable interval. As shown in figure 2A,C, the coincidence rate was 86.49%, and r^2 was 0.7632 in the range of 0.36–1.50 g/L. The coincidence rate was 86.21% and r^2 was 0.2270 in the range of 8.01–10.22 g/L.

Practical application

Based on the validation results, a practical protocol and workflow for Fib evaluation were established in our laboratory as follows: (1) all Fib samples were initially screened using the recalibrated PT-Fib method in an automated analyzer; (2) if the results fell into the range of 1.51–8.00 g/L and the underlying patient conditions associated with bias were not present, the results will be released directly; (3) for patients with the underlying conditions mentioned above, a manual request of Clauss fib assay on the automated analyzer will be used to retest the Fib concentration for clinical reporting; (4) if the results of the recalibrated PT-Fib method did not fall in the range of 1.51–8.00 g/L, the Clauss method will be used for the reflex testing. After this procedure was applied in the second half of the year, Fib retesting rate in our laboratory dropped to just 2.69% (295/10975), which was much lower than 35.65% (3913/10975) estimated from the original manufacturer guidelines. At the same time, the detection speed and cost-effectiveness were also improved.

DISCUSSION

Earlier studies have identified several factors that may contribute to the artificially elevated fibrinogen results obtained from the PT-Fib test. Except for the genetically determined structural variation in the proteins themselves, the other two significant factors were insufficient value transfer on calibration and matrix effects linked to the underlying circumstances of the patient. The selection of calibrator or standard is very important for PT-Fib as the assay is an indirect measurement of Fib based on optical change. It is difficult to achieve calibration if the reference plasma has a high fibrinogen level or excess turbidity.[13 17] Pooled fresh human plasma of good optical quality is recommended for calibration purpose, because it is free of reconstitution errors,

Table 3 Comparison between Clauss and recalibrated PT-Fib method in different range

PT-Fib results range (g/L)	Sample numbers	Results $\bar{x}\pm s$ (g/L)		Bias $\bar{x}\pm s$ (%)	Accordance rate (%)
		PT-Fib	Clauss		
0.36–1.00	25	0.76±0.18	0.85±0.20	−9.66±15.62	64.00
1.01–1.50	86	1.30±0.14	1.39±0.20	−5.88±11.93	93.02
1.51–2.37	745	2.06±0.23	2.19±0.30	−4.96±8.39	99.60
2.38–4.98	3639	3.40±0.69	3.40±0.71	0.35±6.80	99.78
4.99–8.00	691	5.93±0.78	5.86±0.86	1.61±6.18	99.42
8.01–10.22	29	8.49±0.57	8.19±0.75	4.16±8.94	86.21

PT-Fib, prothrombin time-derived fibrinogen.

Table 4 Comparison between Clauss and recalibrated PT-Fib method in another ACL TOP300

PT-Fib results Range (g/L)	Sample numbers	Results $\bar{x}\pm s$ (g/L)		Bias $\bar{x}\pm s$ (%)	Accordance rate (%)
		PT-Fib	Clauss		
1.51–7.93	503	3.46±1.13	3.46±1.09	4.80±4.13	99.60

PT-Fib, prothrombin time-derived fibrinogen.

mimics patient specimens more closely and produces superior intra-assay precision.[24]

To further improve PT-Fib standardisation, in the present study, an intralaboratory reference value transfer was implemented. To derive a traceable PT-Fib reference material, freshly pooled human plasma samples were value assigned by the Clauss assay, which is the reference method recommended by WHO. Then, the analyzer was calibrated by performing a PT time test on this reference plasma and the optical change in each test sample was then converted to a fibrinogen value. The precision analysis demonstrated that the recalibrated PT-Fib method fulfils the requirements claimed by PT-Fib assay manufacturer. At the same time, the linear analysis using five gradient dilution controls showed that the recalibrated PT-Fib method can detect Fib with a clinically acceptable third-order linearity in the range of 1.27–8.00 g/L.

Nevertheless, matrix effects of patient samples also create discrepancies between the PT-Fib and Clauss methods. As a functional reactant, fibrinogen concentration changes in response to both acute and chronic processes. Especially in samples from patients with disseminated intravascular coagulation, liver disease, renal disease, dysfibrinogenaemia, those receiving anticoagulants or thrombolytic therapy, fibrinogen levels will increase in response to inflammation.[2 3] In our study, we found that even after the recalibration by pooled plasma, the clinically unacceptable bias (≥20%) still could be observed in patients that had experienced extensive anticoagulant therapy, had been diagnosed with liver sclerosis decompensation or had some other coagulation disorder with elevated levels of FDP, TT or APTT. Rizzo *et al* also found that the deviations detected in different methods in fibrinogen levels are related to the severity of cirrhosis,[25] which is consistent with our results that the detection value deviation of the two methods is over 20% in cirrhosis decompensation period. In this case, whether the Clauss method is more accurate than the PT-Fib method is still under discussion. Even though more than 99% results between these two methods still can be interchanged, we found a strong consistency between these two tests, with concordance rates>99% in the range of 1.51–2.37 g/L, 2.38–4.98 g/L and 4.99–8.00 g/L. In addition, under values of 1.51–8.00 g/L, linear regression analysis also revealed that more than 99% of values in our study were interchangeable between these two methods. In summary, we examined the agreement between the automated Clauss and PT-Fib methods, in which the PT-Fib method was recalibrated using freshly pooled human plasma in our laboratory.

This recalibration protocol has also been applied to another ACL TOP300 instrument in our hospital. In the interval of 1.51–7.93 g/L (table 4, figure 1C,D), 501 of 503 results met the requirements for consistency (bias≤20%, between the two plotted lines) and accordance rates was 99.60%. In contrast, the bias exceeded the criteria in the wider ranges between 0.77 and 11.45 g/L (table 1, figure 1A,B).

The findings from this study indicated that these two methods are interchangeable for a broad range of patients without certain underlying conditions. As compared with a protocol using the Clauss method, we also showed that the recalibrated PT-Fib decreased the reflex testing and improved cost-effectiveness of Fib testing. The PT reagents of different product batches need to be recalibrated and the calibration results are only suitable on certain instruments. Therefore, recalibration may also be required on a per-instrument basis. Other laboratories can refer to this protocol for recalibration and validation.

Take home messages

- The recalibration of prothrombin time-derived fibrinogen (PT-Fib) is carried out completely according to the value transfer method and can be traced to the reference material.
- The performance of the calibrated PT-Fib method meets the standards.
- The recalibrated PT-Fib method can almost replace the Clauss method in the range of 1.51–8.00 g/L.
- The manufacturer's guideline suggested that the PT-Fib result should be reviewed by Claus method when the result is beyond 2–4 g/L, with a review rate of 35.65%. Based on the results of this study, the Claus method only needs to be reviewed outside the range of 1.51–8.00 g/L and in some rare cases, with a reduced review rate of only 2.69%. Therefore, the recalibrated PT-Fib test resulted in significant savings in reagent and time costs.

Handling editor Mary Frances McMullin.

Acknowledgements The authors would like to thank Xuejuan Chen of Coag-South Central, Werfen for her product support.

Contributors SW, XD and XJ conceived and designed research. SW, XD and XL collected data and conducted research. SW, LW, YZ and XJ analysed and interpreted data. SW wrote the initial paper. SW, XD and XL revised the paper. SW had primary responsibility for final content. All authors read and approved the final manuscript.

Funding This study was supported by 2019 Three Gorges University Youth Science Foundation Project (No. KJ2019A004).

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. The datasets generated and analysed during the present study are available from the corresponding author on reasonable request.

ORCID iD
Sheng Wang http://orcid.org/0000-0002-5958-1212

REFERENCES

1 Song W. Evaluation and application of two methods for determination of fibrinogen by ACL7000 blood coagulation instrument. *Int Lab Med* 2016;37:2493–4.
2 Lupi A, Secco GG, Rognoni A, *et al*. Plasma fibrinogen levels and restenosis after primary percutaneous coronary intervention. *J Thromb Thrombolysis* 2012;33:308–17.
3 Zhou Z-M, Yang S, Yue X-Y, *et al*. [Influencing factors for cerebral microbleeds in patients with acute ischemic stroke]. *Zhonghua Yi Xue Za Zhi* 2010;90:451–3.
4 Meade TW. Fibrinogen in ischaemic heart disease. *Eur Heart J* 1995;16(Suppl A):31–5. discussion 4-5.
5 Feng D, D'Agostino RB, Silbershatz H, *et al*. Hemostatic state and atrial fibrillation (the Framingham offspring study). *Am J Cardiol* 2001;87:168–71.
6 Alessandri C, Basili S, Vieri M, *et al*. [Relationship between blood fibrinogen and plasma lipoproteins in patients with hypercholesterolemia]. *Minerva Med* 1994;85:287–92.
7 Hijazi Z, Oldgren J, Siegbahn A, *et al*. Biomarkers in atrial fibrillation: a clinical review. *Eur Heart J* 2013;34:1475–80.
8 Ang L, Mahmud E. Elevated serum fibrinogen: an independent link between diabetes mellitus, impaired on-clopidogrel platelet inhibition, and major adverse cardiac events after percutaneous coronary intervention. *J Am Coll Cardiol* 2015;65:1713–4.
9 Chen T. Comparison of two methods detecting fibrinogen on Sysmex CA7000. *Int Lab Med* 2015;36:1546–7.
10 Lawrie AS, McDonald SJ, Purdy G, *et al*. Prothrombin time derived fibrinogen determination on Sysmex CA-6000. *J Clin Pathol* 1998;51:462–6.
11 Szefler SJ, Baker JW, Uryniak T, *et al*. Comparative study of budesonide inhalation suspension and montelukast in young children with mild persistent asthma. *J Allergy Clin Immunol* 2007;120:1043–50.
12 CLSI. *H30-A, procedure for the determination of fibrinogen in plasma; Approved guideline*. 99. Wanye, PA: CLSI, 1999.

13 Mackie IJ, Kitchen S, Machin SJ, *et al*. Guidelines on fibrinogen assays. *Br J Haematol* 2003;121:396–404.
14 Nieuwenhuizen W. Biochemistry and measurement of fibrinogen. *Eur Heart J* 1995;16(Suppl A):6–10. discussion 10.
15 Llamas P, Santos AB, Outeiriño J, *et al*. Diagnostic utility of comparing fibrinogen Clauss and prothrombin time derived method. *Thromb Res* 2004;114:73–4.
16 Miesbach W, Schenk J, Alesci S, *et al*. Comparison of the fibrinogen Clauss assay and the fibrinogen PT derived method in patients with dysfibrinogenemia. *Thromb Res* 2010;126:e428–33.
17 Mackie J, Lawrie AS, Kitchen S, *et al*. A performance evaluation of commercial fibrinogen reference preparations and assays for Clauss and PT-derived fibrinogen. *Thromb Haemost* 2002;87:997–1005.
18 Shang H, Wang YS, Shen ZY. *National guide to clinical laboratory*. 4th edn. Procedures People's Health Publishing House, 2015.
19 Wang S, Duan XY, Liu XT, *et al*. Establishment and practice of quality management system for clinical POCT testing project. *J Mod Lab Med* 2019;34:147–52.
20 NCCLS. *User demonstration of performance for precision and accuracy; Approved guideline. NCCLS document EP15-A*. Wayne, PA: NCCLS, 2001: 1–48.
21 NCCLS. *Evaluation of the linearity of quantitative measurement procedures: a statistical approach; Approved guideline. NCCLS document EP6-A*. Wayne, PA: NCCLS, 2004: 1–47.
22 NCCLS. *How to define and determine reference intervals in the clinical laboratory; Approved guideline, NCCLS document C28-A2*. 2nd edn. Wayne, PA: NCCLS, 2000: 1–38.
23 YS L, Liang HQ, Han XJ, *et al*. Analytical performance of high-sensitivity cardiac troponin I detection by VITROS ECIQ with enhanced chemiluminescence method. *Chin J Lab Med* 2015;38:461–4.
24 Tong Q, Chen BR, Wang QT. Study on the quantity value transfer of enzymes from the reference measurement system. *Chin J Lab Med* 2014;37:110–4.
25 Rizzo K, Vella K, Zammit D, *et al*. Fibrinogen measurement in liver disease: validation of the functional fibrinogen thromboelastography assay and a novel mathematical predictive model. *Blood Transfus* 2019;17:237–46.