Original Article

Performance verification of a new fibrinogen assay

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Background: Plasma fibrinogen (FIB), also known as factor I, plays a key role in the coagulation process. FIB testing in a clinical laboratory is crucial for coagulation screening and thrombolytic therapy. Here, we assessed the performance of a new, Chinese-made coagulation analyzer in the detection of FIB by comparing its precision and clinical feasibility with that of an imported system.

Methods: Blood samples were collected and plasmas were separated. The precision, linearity, reference interval, carryover rate, clinically reportable range, and clinical applicability of the domestic coagulation analyzer for FIB assay were assessed and validated based on the documents or industry standards issued by the United States Clinical and Laboratory Standards Institute (CLSI).

Results: The within-batch precision CVs (coefficient of variation) for the low- and high-level specimens were 2.92% and 0.24%, respectively, while the total precision CVs were 3.05% and 1.81%, respectively; all of them met the experimental requirements. The linear range was validated to cover 1.0–6.5 g/L, and a good linear relationship was obtained within the measurement range (R^2=0.9998). The reference interval was verified for adults and the carryover rate was also evaluated to be 0.68%. The clinically reportable range was 0.33–13.0 g/L. With a sample size of 180 cases, the methodological comparison showed a correlation coefficient (r) of 0.9886 between Mindray ExC810 and Sysmex CS5100. Furthermore, when the level of FIB was higher than 4.0 g/L or lower than 1.0 g/L, the 2 systems had an agreement rate of 100%.

Conclusions: Mindray ExC810 has a good performance for FIB assay in terms of precision, linearity, reference interval, carryover rate, and clinically reportable range. Methodological study showed that Mindray ExC810 has good agreement with Sysmex CS5100 and meets the requirements of laboratory testing. Therefore, Mindray ExC810 is suitable for FIB assay in clinical laboratory.

Keywords: Fibrinogen; domestic coagulation analyzer; performance verification

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Introduction

Fibrinogen (FIB, also called factor I), which is synthesized in the liver, is the most abundant coagulation factor in blood plasma. The normal plasma FIB level in adults ranges from 200–400 mg/dL, with a half-life of 3–5 days and a molecular mass of 340 kD (1). FIB is made up of 2 identical subunits, each comprising 3 polypeptide chains—α, β, and γ—which are interconnected by as many as 29 disulfidisulfide bonds. The N termini of these 3 peptide chains are intertwined, forming the E region, a helical coiled-coil structural domain, in which the aggregated disulfide bonds are essential for the maturation of Fg molecules (2,3). The C-termini of the β and γ chains form the D region, while the C-terminal of the α chain folds back to participate in the structure of E region. The D and E regions are connected by a band structure (called a "coiled coil") formed by 3 peptide chains through the α-helix. Lord *et al.* (4) and Weisel *et al.* (5) reported that the structure and stability of fibrin clots have a powerful effect

on the function of FIB. During its transformation into fibrin clots, FIB demonstrates anti-thrombogenic properties by exposing its non-substrate thrombin-binding sites (so-called antithrombin I activity) (6). FIB is a key player in the process of hemostasis and has direct involvement in the coagulation process as coagulation factor I (7). FIB also connects with heparin, fibronectin, and cell adhesion molecules, and participates in cell–matrix interactions, playing roles in cell proliferation, angiogenesis, wound healing, and tumor cell metastasis (8). Different coagulation analysis items have different factors affecting accuracy. For FIB assay, the principle of measurement (Clauss assay, PT-derived fibrinogen) and traceability, instrument performance (precision, measurement range), reagent performance (linearity, stability, inter batch difference), research sample characteristics (distribution range, drug type) can affect the determinated accuracy of FIB.

We present the following article in accordance with the MDAR checklist (available at http://dx.doi.org/10.21037/apm-21-298).

Methods

Materials

Specimens

Plasma specimens were collected from 180 inpatients and outpatients (95 males and 85 females) of Handan Municipal Central Hospital between January 2020 and May 2020. The sample size of 180 met the requirements of CLSI EP9-A3 (more than 100 cases). All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). Ethical approval for the study was granted by the ethics committee of Handan Municipal Central Hospital with the approval number being (2019) LSX No. (005). Written informed consent was exempted.

Equipment and reagents

For the Mindray ExC810 group, an automatic coagulation analyzer was purchased from Shenzhen Mindray Corporation (model: Mindray ExC810). The supporting reagents Fibrinogen (FIB) assay kits (coagulation method) were obtained from Long Island (LongIsland Biotech, Shanghai, China) (lot no.: 200809700), and a fibrinogen calibrator (lot no.: 200809700) was used for calibration. For the control group, an automatic coagulation analyzer was purchased from Sysmex Shanghai Ltd. (model: CS5100), and the supporting reagents Dade Thrombin Reagent kits were obtained from Siemens (Siemens Healthcare Diagnostics, Beersel, Belgium) (lot no.: 565026).

Research methods

Precision test

Total precision: According to EP15-A2 by the Clinical and Laboratory Standards Institute (CLSI) (9), 2 levels of quality control plasma were prepared. For each level, 3 replicates were performed as a batch per day. The experiment lasted for a total of 5 days and also included daily quality control. In case of failures for quality control or errors in operation, the results of the batch would be rejected and those of another batch would be introduced. The mean, standard deviation, and coefficient of variation were calculated for each level to obtain the within-batch and total precision. According to CLIA '88 (the Clinical Laboratory Improvement Amendments of 1988), the within-batch precision should be no more than 1/4 of the allowable total error (TEa). The total precision needed to no more than 1/3 of TEa, and if it was not specified in CLIA '88, the corresponding data described in the reagent instructions should be followed or verified as well as the requirements of external quality assessment (EQA) organized by the Ministry of Health of China.

Within-batch precision: According to the current domestic industry standards, 20 consecutive tests of the validation materials (mixed fresh plasmas or quality control products) were performed in one batch, and the CVs calculated for both levels should be less than the declarations in the manufacturer's instructions.

Linear range determination

According to CLSI EP6-A (10), low- and high-value plasma specimens (at concentrations to cover the linear range specified in the reagents instructions as far as possible) were collected. The high values were expected to reach the upper limit of the linear range declared by the manufacturer, and the low values closest to the lower limit. The same reagents as well as calibrators were used throughout the evaluation course. A series of sample concentrations were prepared by mixing low- (L) and high-value (H) plasma in certain proportions (5L, 4L+1H, 3L+2H, 2L+3H, 1L+4H, and 5H). Two replicates of each sample were tested on the analyzer. The mean values were calculated, and regression analysis was conducted to compare the measured and expected values. The regression and correlation coefficients

were calculated. The expected values (X) of the diluted samples were determined by the average slope method and the measured values (Y) adopted the mean values of repeated tests. All the results were plotted on an XY-coordinate graph. Different analyzers have different linear ranges, but industry-recognized standards require all analyzers to have: (I) linearly distributed results throughout the specified linear range; and (II) a linear correlation coefficient of more than 0.99.

Validation of reference intervals

According to CLSI EP28-A3c (11) (*How Clinical Laboratories Determine and Establish Biological Reference Intervals*), 20 specimens collected from healthy individuals who had received health check-ups were tested with the analyzer to obtain FIB concentrations. The results were compared to the reference intervals suggested by the manufacture. On condition that less than 2 of the 20 specimens fell out of the reference interval suggested by the manufacture, the validation was regarded as "passed". Otherwise, a reference interval had to be established by the local laboratory.

Validation of carryover rate

According to China industry standard (12), plasma samples of high (H) and very low (L) concentrations were prepared. The very low concentration plasma was divided into 4 tubes (L1, L2, L3, L4) and the high concentration plasma was divided into 3 tubes (H1, H2, and H3). The 7 tubes were continuously analyzed in the following order: L1, L2, L3, H1, H2, H3 and L4. Then, the carryover rate was calculated.

$$Carryover = \frac{L4 - (L1 + L2 + L3)/3}{H3 - (L1 + L2 + L3)/3} \times 100\% .$$

Clinically reportable range

Specimens with a concentration around 90% of the upper limit were collected. 2-fold dilution of the specimens were conducted and 3 replicates of each diluted sample were tested on the analyzer. The mean value of each diluted sample was calculated as the diluted concentration and compared with the theoretical concentration. The deviation between the mean diluted value and the theoretical concentration was calculated. The optimal deviation was supposed to be less than 1/2 of the TEa. Among all the diluted samples those met the requirement, the maximum concentration was selected. The corresponding value was multiplied by 2 to determine the upper limit of the clinically reportable range (CRR).

Methodological study

A total of 180 fresh specimens that met the required sample size (more than 100 cases) by CLSI EP9-A3 were collected tested. An evenly distribution of sample concentration was recommended to within the linear range. The specimens were easily obtained in clinical laboratory and properly stored to keep stable before measurement. The specimens were analyzed on the Mindray ExC810 system, and linear fit analysis with the results of the control group (in which the Sysmex CS 5100 system was used) was performed. The result was "Y = aX + b", with an R^2 of >0.95 required compared with the Sysmex system. The EP9-A3 states that if the requirement cannot be met, the sample scale can be expanded or partitioned for bias analysis. The elimination of outliers is conducted by calculating the average difference between the datasets of the 2 groups (Y-X); if the difference of one paired result exceeds the average by 4 times, the sample data is considered as an outlier.

Statistical analysis

The results were analyzed by linear regression analysis. Bland-Altman plot was also introduced to make bias analysis, with the mean results of the 2 methods as x-axis and the difference between them as y-axis.

Results

Results of precision test

The within-batch precision CVs of the Mindray analyzer were 2.92% and 0.24% at level 1 (2.68 g/L) and level 2 (5.30 g/L), respectively. The total precision CVs for levels 1 and 2 were 3.05% and 1.81%, respectively (*Table 1*).

Results of linear range determination

The low-concentration specimens (0.70 g/L, abbreviated as L) and the high-concentration specimens (6.65 g/L, abbreviated as H) were mixed in certain proportions to prepare 6 different dilutions of plasma, which were subsequently used for linear experiments. The preparation methods and the experimental results are summarized in *Table 2*. *Figure 1* shows the results of the linear regression analysis.

Results of validation of reference intervals

Blood specimens were randomly collected from 20 healthy individuals (10 males and 10 females aged 20 to 70 years).

Table 1 Within-batch precision CV and total precision CV of the Mindray analyzer

Specimen	Mean ($\bar{X}$) (g/L)	Inter-day precision SD	Within-batch precision		Total precision	
			SD	CV (%)	SD	CV (%)
Level 1	2.68	0.057	0.08	2.92	0.08	3.05
Level 2	5.30	0.018	0.01	0.24	0.09	1.81

SD, standard deviation; CV, coefficient of variation.

Table 2 Theoretical concentrations and experimental results in the linear analysis

Dilution ratio (L:H)	Theoretical concentration (g/L)	Measured value (g/L)		Average value (g/L)
		1st	2nd	
5:0	0.70	0.68	0.72	0.70
4:1	1.89	1.68	1.72	1.70
3:2	3.08	2.68	2.7	2.69
2:3	4.27	4.04	4.19	4.115
1:4	5.46	5.67	5.62	5.645
0:5	6.65	6.67	6.65	6.66

L, low; H, high.

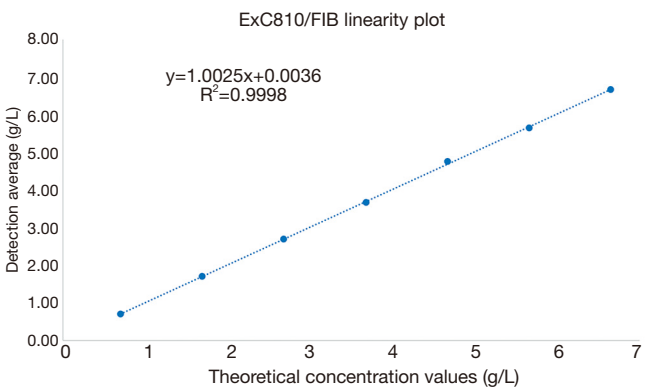

Figure 1 Linear regression analysis plot.

The specimens were calibrated and subjected to quality control with Mindray's original reagents, and the qualified specimens were analyzed. In all 20 subjects, the results were in the range of 2–4 g/L. Thus, the requirement that there should be no more than 2 specimens outside the range was met, and the reference interval passed the verification.

Results of validation of the carryover rate

The results of validation of the carryover rate in 6 samples are shown in *Table 3*. $Carryover = \frac{L4-(L1+L2+L3)/3}{H3-(L1+L2+L3)/3}\times 100\%$. The carryover rate was 0.68%.

Results of verification of the CRR

The results of verification of the CRR of the Mindray analyzer are shown in *Table 4*, and the CRR was 0.33–13.0 g/L.

Results of methodological study

Methodological comparison revealed that Mindray ExC810 showed good agreement with Sysmex CS5100 (r=0.9986) and met the requirements of laboratory testing (*Figure 2*, *Table 5*).

Discussion

Sysmex CS5100 is the most widely used coagulation analyzer in China, and also the main model of our hospital. The comparison with Sysmex CS5100 is an important way to evaluate the clinical applicability of the new domestic coagulometer in Chinese population. FIB is an acute phase protein. Physiological stimuli, such as pregnancy, immune response, or estrogen use, can trigger an increase in plasma FIB levels. Under normal physiological conditions,

Table 3 Results of validation of the carryover rate

Variable	Outcome
Results analysis	0.95/0.92/0.89, 5.37/5.29/5.33, 0.95
Carryover rate	0.68%

Table 4 Results of verification of the clinically reportable range

Specimen	2-fold dilution	Original concentration
Test 1	6.38	6.23
Test 2	6.45	5.86
Test 3	6.25	6.34
Theoretical concentration	5.92	
Average value	6.36	6.14
CV (%)	0.251	1.032%
Bias	3.583%	
Reportable range (g/L)	0.33–13.0	

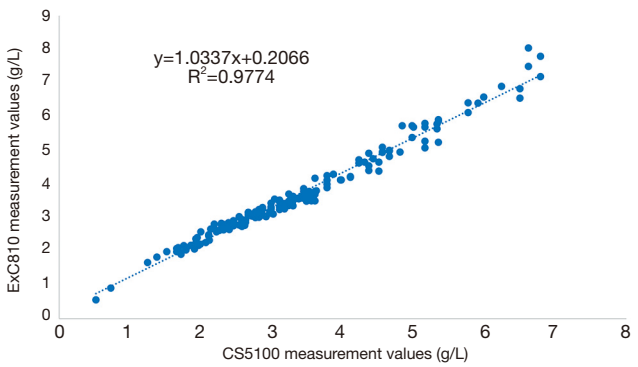

Figure 2 Results of methodological comparison between Mindray ExC810 and Sysmex CS5100.

Furthermore, a high level of FIB may be a risk factor for the development of venous or arterial thrombotic complications. Therefore, FIB can serve as a marker for assessing the risk of thrombotic complications (13). A reduction in FIB can be seen in patients with severe liver disease, although it is mostly observed after the use of thrombolytic or fibrin-lowering drugs. In October 1999, the National Conference on Thrombosis and Haemostasis identified the indicators of bleeding risk in patients receiving systemic thrombolytic therapy, and a FIB level of less than 1.0 g/L was the preferred risk indicator (14).

Clinically, FIB can be detected by using a variety of techniques such as the biuret method, the immunoturbidimetric method, and the prothrombin time test. In our current study, we investigated the performance of a Chinese-made coagulation analyzer (Mindray ExC810) for FIB assay, with a focus on precision, linearity, reference interval, CRR, carryover rate, and methodological comparison. The Mindray ExC810 showed good within-batch and total precision at both low and high levels; the CRR was 0.33–13.0 g/L based on a maximum of 2-fold dilution, and a good linear relationship within the measurement range was observed. The methodological comparison showed Mindray ExC810 is competent for clinical laboratory use, with a good correlation coefficient of 0.9886 to Sysmex CS5100. Moreover, the analyzer showed a high degree of consistency with Sysmex CS5100 in the interpretation of the clinical diagnostic results.

The new domestic coagulometer in this study is a measuring instrument based on magnetic bead method, thus lacking PT-derived fibrinogen as a comparison of Clauss method. However, this is not only a shortcoming of domestic instruments, but also a challenge for the whole industry.

In summary, the Mindray ExC810 is an automatic, efficient, and accurate system for FIB assay and has high

Table 5 Results of the comparison between the 2 analyzers

Concentration (g/L)	Sysmex CS5100 (n)	Mindray ExC810 (n)	Agreement of ExC810
<1.0	2	2	100.00%
1.0:2.0	20	19	95.00%
2.0:4.0	116	117	99.15%
>4.0	42	42	100.00%

the fibrinolytic and coagulation systems work in dynamic balance; however, abnormalities in the function of either system can lead to bleeding or thrombotic disease. An elevated FIB level is closely associated with cardiovascular diseases and thrombosis, and an FIB level that is persistently high may increase the risk of venous or arterial thrombosis.

agreement rate with the reference system.

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Footnote

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Data Sharing Statement: Available at http://dx.doi.org/10.21037/apm-21-298

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/apm-21-298). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). Ethical approval for the study was granted by the ethics committee of Handan Municipal Central Hospital with the approval number being (2019) LSX No. (005). Written informed consent was exempted.

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