RESEARCH ARTICLE

Performance evaluation of thrombus molecular markers thrombomodulin, thrombin-antithrombin complex, plasmin-α2-plasmin inhibitor complex, and tissue plasminogen activator-inhibitor complex by a chemiluminescence analyzer

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Abstract

Objective: To comprehensively evaluate the primary performance regarding the task of detecting thrombomodulin (TM), thrombin-antithrombin complex (TAT), plasmin-α2-plasmin inhibitor complex (PIC), and tissue plasminogen activator/plasminogen activator inhibitor-1 complex (t-PAIC) by Sysmex HisCL5000 high sensitivity chemiluminescence analyzer.

Methods: The performance of the chemiluminescence analyzer was evaluated according to the Clinical and Laboratory Standards Institute (CLSI) documents for in-batch precision, daytime precision, carryover rate, linearity, and reference range.

Results: The intra-batch and inter-day variation coefficients of the test items were all less than 5 %, and the contamination rate of each index was less than 10 %. The linear verification analysis showed that the correlation coefficients of TM, TAT, PIC, and t-PAIC were 0.9968, 0.9988, 0.9981, and 0.9930, respectively. The project recommended reference range was applicable to our laboratory.

Conclusion: The high-sensitivity chemiluminescence analyzer has good performance in the detection of TM, TAT, PIC, and t-PAIC and is suitable for the detection of clinical specimens. HIPPOKRATIA 2022, 26 (2):78-82.

Keywords: High sensitivity chemiluminescence analyzer, performance evaluation, thrombus molecular markers, thrombomodulin, thrombin-antithrombin complex, plasmin-α2-plasmin inhibitor complex, tissue plasminogen activator-inhibitor complex

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Introduction

Thrombomodulin (TM) is a membrane-bound receptor for thrombin on the surface of endothelial cells, which is well involved in regulating blood coagulation, innate immunity, inflammation, and cell transport. TM is a marker of vascular endothelial system injury[1]. Thrombin-antithrombin complex (TAT) is a complex composed of thrombin and its inhibitor in equal proportion, which is mainly used as a marker for initiating the blood coagulation system. The plasmin-α2-plasmin inhibitor complex (PIC) is a marker for initiating the fibrinolytic system. Tissue plasminogen activator-inhibitor complex (t-PAIC) can determine the degree of repair of the vascular endothelial system and is a marker of the fibrinolytic system. Compared with conventional coagulation items such as prothrombin time (PT) and activated partial thromboplastin time (APTT), thrombus molecular markers (TM, TAT, PIC, t-PAIC) are all generated at the initial stage of response in each system, which can be more sensitive and comprehensive to evaluate the occurrence and development of thrombus and monitoring the efficacy of thrombolysis for postoperative thrombus and bleeding, and vascular endothelial system injury monitoring. The characteristics of the Sysmex HisCL5000 high-sensitivity chemiluminescence analyzer are fast detection speed and high sensitivity. It has been used in the performance evaluation of hepatitis B markers, carcinoembryonic antigen, abnormal prothrombin (PIVKA-II), and other projects[2-4]. However, performance verification of TM, TAT, PIC, and t-PAIC is rare, and the laboratory needs to evaluate the effective performance and indicators of newly purchased instruments. Therefore, we evaluated the performance of the chemiluminescence analyzer in detecting TM, TAT, PIC, and t-PAIC according to CLSI documents, including in-batch precision, daytime precision, carryover rate, linearity, and reference range to ensure the effectiveness and reliability of the inspection results.

Materials and Methods

Specimen Source

The study was designed to follow the principles of current legislation and regulations and was approved by

the Ethics Committee of the First Hospital of Lanzhou University (decision No: LDYYLL-2023-247). The evaluation samples were the plasma of healthy physical examinators from the First Clinical Medical College of Lanzhou University. The samples of healthy subjects and patients were fresh venous blood containing sodium citrate anticoagulant (sodium citrate: blood volume = 1: 9), and the blood volume was 2.7 mL. The centrifugation was performed at 3000 r/min for ten min, and the test was completed within two hours. Dissolve the quality control product with pure water, and store it at 2-8 ℃ after measurement.

Instruments and Reagents

A Sysmex Hiscl5000 (Siemens, Kobe, Japan) high-sensitivity chemiluminescence analyzer and its associated reagents and quality control products were used to test the samples according to the manufacturer's instructions. The total test time was 17 minutes.

Methods

Precision evaluation

Intra-day precision was evaluated based on the requirements of CLSI EP15-A2[5]. The specific methods are as follows, one quality control product at two concentration levels was taken, and each sample was repeatedly tested ten times according to the conventional method, and the coefficient of variation can be determined by applying Equation 1:

$$CV = s / \bar{x} \quad \text{(Equation 1)}$$

where *CV* is the coefficient of variation, $\bar{x}$ is the mean, and *s* is the standard deviation, respectively. The final result was judged by *CV* less than 5 %. Similarly, the quality control substances with two concentration levels were taken during the inter-day for precision. Each concentration was repeated four times, and consecutive testing five days with the same batch number of reagents. The results of TM, TAT, PIC, and t-PAIC were recorded during the test. The coefficient of variation was calculated according to the relation of Equation 1, and *CV* less than 10 % was set as the criterion.

Carried contamination rate

According to the requirements of CLSI H57-A[6], high-concentration plasma samples were taken, mixed evenly, and continuously measured three times, with the measured values of H1, H2, and H3, respectively. Then low-concentration plasma samples were taken and determined three consecutive times with the measured values of L1, L2, and L3, respectively. The carried contamination rate calculation formula was obtained:

$$\text{Carried contamination rate} = \frac{L1 - L3}{H3 - L3} \times 100\% \quad \text{(Equation 2)}$$

Linear evaluation

A sample with a high value close to the expected upper limit was selected based on the requirements of CLSI EP6-A[7] and diluted with diluent at the proportions of 100 %, 80 %, 60 %, 40 %, 20 %, and 0 %. Each dilution degree was repeated three times. The regression equation Y = aX + b was obtained by taking the theoretical value as the independent variable and the measured value as the variable. The experimental results should meet specific requirements, namely a value in the range of 1 ± 0.05 correlation coefficient $R^2 \geq 0.95$.

Reference interval evaluation

Ten male and ten female specimens of healthy subjects were randomly collected according to the CLSI C28-A[8]. Continuous measurement was carried out on the machine under the stable condition of the instrument, and all the report parameters that provided the reference range were evaluated; 95 % of the test values within the reference range were considered qualified.

Statistics process

Excel software was used to analyze the $\bar{x}$, *s*, and *CV* values of the data, and Origin software (OriginLab Corp., Northampton, MA, USA) was utilized to process the linear and comparison data.

Results

Precision evaluation results

The precision for intra-day and inter-day was less than 5 % of normal and abnormal levels of the quality control plasma detected by HisCL5000, both meeting the requirements of the standard, and the results are shown in Table 1 and Table 2.

Carried contamination rate evaluation results

The carried contamination rate of TM, TAT, PIC, and t-PAIC were 0.12 %, -0.11 %, 0.10 %, and 0.43 %, respectively. The results were within acceptable limits and are given in Table 3.

Linear evaluation results

The regression equation of TM theoretical value and the measured value is $Y = 1.0128x + 1.8714$ with $R^2 = 0.9968$, the TAT regression equation is $Y = 1.0086x + 0.6431$ with $R^2 = 0.9988$, the PIC regression equation is $Y = 0.9879x + 0.3667$ with $R^2 = 0.9981$, and the t-PAIC regression equation is $Y = 0.9897X - 0.9804$ with $R^2 = 0.9930$. They all meet the requirements of a value within the range of 1 ± 0.05 and correlation coefficient $R^2 \geq 0.95$, as shown in Figure 1.

Reference interval evaluation results

The TM, TAT, PIC, and t-PAIC test values of 20 healthy subjects in this area are consistent with the reference interval provided by the manufacturer, as shown in Table 4.

Discussion

Thromboembolic diseases include mainly venous thromboembolic diseases (pulmonary thromboembolic syndrome deep vein thrombosis) and arterial thromboem-

Table 1: Intra-day precision results regarding the chemiluminescence analyzer performance based on the Clinical and Laboratory Standards Institute EP15-A2 document requirements.

Test items	Low concentration			High concentration		
	$\bar{x}$	*s*	*CV*/%	$\bar{x}$	*s*	*CV*/%
TM (TU/mL)	20.44	0.37	1.79	78.83	1.01	1.28
TAT (ng/mL)	10.28	0.18	1.76	40.40	0.36	0.88
PIC (μg/mL)	2.01	0.05	2.48	7.33	0.17	2.31
t-PAIC (ng/mL)	4.79	0.10	2.07	18.09	0.28	1.55

TM: thrombomodulin, TAT: thrombin-antithrombin complex, PIC: plasmin-α2-plasmin inhibitor complex, tPAIC: tissue plasminogen activator/plasminogen activator inhibitor-1, CV: coefficient of variation, $\bar{x}$: mean, s: standard deviation.

Table 2: Inter-day precision results regarding the chemiluminescence analyzer performance based on the Clinical and Laboratory Standards Institute EP15-A2 document requirements.

Test items	Low concentration			High concentration		
	$\bar{x}$	*s*	*CV*/%	$\bar{x}$	*s*	*CV*/%
TM (TU/mL)	20.38	0.55	2.68	78.28	1.61	2.06
TAT (ng/mL)	9.97	0.32	3.23	40.16	0.91	2.27
PIC (μg/mL)	1.98	0.07	3.69	7.16	0.18	2.53
t-PAIC (ng/mL)	4.81	0.07	1.41	18.32	0.32	1.73

TM: thrombomodulin, TAT: thrombin-antithrombin complex, PIC: plasmin-α2-plasmin inhibitor complex, tPAIC: tissue plasminogen activator/plasminogen activator inhibitor-1, CV: coefficient of variation, $\bar{x}$: mean, s: standard deviation.

Table 3: Carries contamination rate results regarding the chemiluminescence analyzer performance based on the Clinical and Laboratory Standards Institute H57-A document requirements.

Test items	L1-L3	H3-L3	Carried contamination rate (%)
TM	0.20	165.40	0.12
TAT	-0.10	92.30	-0.11
PIC	0.02	19.40	0.10
t-PAIC	0.20	46.70	0.43

TM: thrombomodulin, TAT: thrombin-antithrombin complex, PIC: plasmin-α2-plasmin inhibitor complex, tPAIC: tissue plasminogen activator/plasminogen activator inhibitor-1.

Table 4: Reference interval result regarding the chemiluminescence analyzer performance based on the Clinical and Laboratory Standards Institute C28-A document requirements.

Test items	Minimum	Maximum	Reference range
TM (TU/mL)	4.5	10.6	3.8~13.3
TAT (ng/mL)	0.3	3.2	＜4.0
PIC (μg/mL)	0.32	0.71	＜0.8
t-PAIC (ng/mL)	female 3.1	female 9.9	female ＜10.5
	male 1.4	male 11.8	male ＜17.0

TM: thrombomodulin, TAT: thrombin-antithrombin complex, PIC: plasmin-α2-plasmin inhibitor complex, tPAIC: tissue plasminogen activator/plasminogen activator inhibitor-1.

bolic diseases (acute coronary syndrome, atrial fibrillation, stroke, etc.), have become one of the leading causes of death worldwide[9]. With medical technology continuously improving, there are progressively more laboratory tests for thrombus and hemostasis in clinical practice. Compared with routine coagulation items such as PT and APTT, the four indicators of thrombus can significantly change at the early stage of coagulation and fibrinolysis systems activation, which is conducive to the early diagnosis and treatment of diseases[10,11]. TM is a type I transmembrane glycoprotein, mainly expressed in vascular endothelial cells, which is an essential cofactor in activating anticoagulant proteins and contributes to hemostatic balance. Specifically, TM regulates thrombin activity from a coagulant to an anticoagulant protease[12]. When thrombin binds to TM, thrombin-activating protein C (APC) selectively inactivates clotting factors Va and VIIIa to prevent excessive clotting. Additionally, TM can enhance the proteolytic activation of thrombin-activated fibrinolytic inhibitors (TAFI), thereby delaying thrombus lysis[13]. In addition to being a specific molecular marker of vascular endothelial injury, TM has been identified as a risk indicator for the progression of atherosclerosis based on research findings[14].

Many studies have demonstrated that administering recombinant soluble thrombo-regulatory protein (rTM) can significantly alleviate bleeding symptoms in patients with DIC, thus indicating its efficacy in treating this condition[15-17]. Thrombin can quickly interact with other components in the bloodstream and become undetectable,

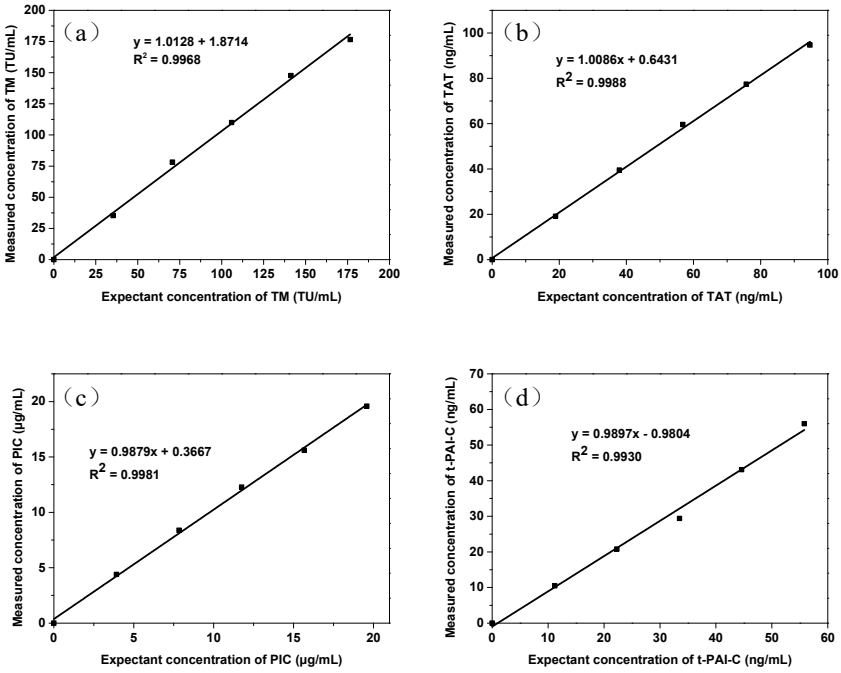

Figure 1: Linear calibration curves based on the Clinical and Laboratory Standards Institute EP6-A document requirements. a) thrombomodulin; b) thrombin-antithrombin complex; c) plasmin-α2-plasmin inhibitor complex; d) tissue plasminogen activator/plasminogen activator inhibitor-1.

TM: thrombomodulin, TAT: thrombin-antithrombin complex, PIC: plasmin-α2-plasmin inhibitor complex, tPAIC: tissue plasminogen activator/plasminogen activator inhibitor-1.

making accurate measurement difficult in clinical settings. However, the thrombin-antithrombin complex (TAT) is a reliable indicator of increased thrombin production and activity in the body, as its level correlates with early coagulation dysfunction. TAT is a complex formed by thrombin binding to antithrombin[18] and exhibits a high degree of sensitivity in the early detection of thromboembolism patients. It can significantly enhance diagnostic accuracy when used in conjunction with D-dimer testing[19].

The fibrinolytic system becomes activated upon the formation of a thrombus in the body. Subsequently, the resulting production of fibrinolytic enzyme binds to specific inhibitors leading to the formation of PIC, which serves as an indicator of hyperfibrinolysis[20]. In a study of 175 cirrhotic patients, TAT and TAT/t-PAIC were identified as potential biomarkers for predicting thrombosis in patients with cirrhosis[21]. t-PAIC is a complex of tissue plasminogen activator (t-PA), and its type 1 inhibitor (PAI-1)[11] is the most important regulator of fibrinolytic and coagulation system balance and elevated plasma PAI-1 levels are closely associated with many cardiovascular diseases[10,22], which has important value in assessing the risk of myocardial infarction and venous thromboembolism.

The enzyme-linked immunosorbent assay (ELISA) is considered the gold standard method for conducting classic TM, TAT, PIC, and tPAI-C tests[23]. However, this technique is time-consuming and has limited reproducibility. In contrast, the Sysmex Hiscl5000 employs chemiluminescent enzyme immunoassay technology, which offers several advantages, including excellent reproducibility, high sensitivity, high specificity, and simplicity of operation. According to guidelines outlined in CLSI files, a chemiluminescent analyzer with high sensitivity was utilized to assess the performance of TM, TAT, PIC, and t-PAIC. The results demonstrated that the instrument exhibits excellent precision and high stability. Furthermore, the linear results revealed that the A value for TM, TAT, PIC, and t-PAI-C fell within the range of 1 ± 0.05, thus satisfying the requisite linearity criteria. Additionally, the correlation coefficient (R^2) was ≥ 0.95, confirming the instrument's analytical accuracy. During the examination of the contamination rate, all values obtained were found to be negligible, which is indicative of the instrument's efficient cleaning system and its ability to prevent cross-contamination between specimens. The evaluation of reference intervals yielded results consistent with those provided by the manufacturer, suggesting that the instrument is less susceptible to regional factors that may impact its performance.

Conclusion

The Sysmex HISCL5000 fully automated coagulation analyzer can accurately measure the performance parameters of TM, TAT, PIC, and t-PAIC, including precision, accuracy, carryover contamination rate, linearity range, and reference values, all of which meet the national healthcare industry standards and manufacturer requirements. This instrument boasts several advantages, such as high automation, rapid detection speed, and low susceptibility to interference, which make it well-suited

for routine use in clinical laboratories, allowing for better meeting clinical demands and providing a reliable basis for the diagnosis and treatment of thrombotic diseases.

Conflict of interest

The authors declare no conflict of interest.

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