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RESEARCH ARTICLE

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Evaluation of a newly developed chemiluminescence immunoassay for detecting cardiac troponin T

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Background: To evaluate the performance of a chemiluminescence detection kit for cardiac troponin T (cTnT).

Methods: According to the "Guiding principles on performance analysis of diagnostic reagents in vitro" and the Clinical and Laboratory Standards Institute (CLSI) Guidelines, we assessed the detection limit, linear range, reportable range, accuracy, precision, cross reactivity, interference factors, and matrix effect of the kit, and compared these parameters with that of the commercial electrochemiluminescence detection kit for cTnT (Roche).

Results: The minimum detection limit of the kit was 0.01 ng/mL. The linear range was 0.01 ng/mL-25 ng/mL. The reportable range was from 0.01 ng/mL to 100 ng/mL. Precision within the batch was 2.9%-6.4%, and precision between batches was 6.0%; the accuracy was good and the recovery rate reached 96.2%. The cross-reaction test demonstrated that there was no reactivity between cTnT and a variety of troponins. Test results deviated by less than ±10% in the presence of hemoglobin ≤1000 μg/mL, bilirubin ≤250 μg/mL, triglycerides ≤11.25 mg/mL, and rheumatoid factor ≤206 U/mL, suggesting that kit results were not significantly interfered with these factors. Results from the matrix-effect assessment revealed absence of a matrix effect in the tested serum samples. Correlation study revealed that the performance of the kit was very consistent with that of the Roche electrochemiluminescence detection kit (Kappa = 0.900, $P < .001$) with a high correlation ($r = .903$, $P < .001$) and a total matching rate of 95.00%.

Conclusion: The performance of the evaluated chemiluminescence immunoquantitation kit for cTnT detection was acceptable in all tested parameters, fulfilling requirements for clinical applications.

KEYWORDS

cardiac troponin T, chemiluminescence immunoassay, evaluation, performance, reagent

1 | INTRODUCTION

Troponin (Tn) is an important regulatory protein necessary for striated muscle contraction. It consists of three subunits: troponin C (TnC), troponin I (TnI), and troponin T (TnT). TnT is the subunit that binds to tropomyosin and is classified into three subtypes: a fast skeletal muscle subtype, a slow skeletal muscle subtype, and a myocardial subtype. Cardiac troponin T (cTnT) is a 37 kD protein that is expressed with a high tissue specificity in myocardium and is approximately 10%-30% structurally different from skeletal troponin. cTnT is present in the blood 3-4 hours after a myocardial infarction, with levels remaining elevated for about 2 weeks. The unique

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molecular structure of cTnT, and the fact that it appears early and lasts longer in the blood upon cardiac muscle damage makes it a highly sensitive and specific marker of myocardial injury and cardiomyocyte death.[1,2] Therefore, cTnT plays an important role in the early differential diagnosis and prognostic evaluation of acute myocardial infarction.[3-6]

At present, an electrochemiluminescence kit developed by Roche is prevalently used for cTnT clinical measurement in China. This kit is highly sensitive and specific for its utilized purpose, but the price of these imported reagents is high. Therefore, developing a domestically produced chemiluminescence immunoassay kit, which possesses independent intellectual property rights, performance stability, and satisfactory precision would greatly reduce the cost for cTnT detection and lighten the financial burden for patients.

In collaboration with Xiamen InnoDx Biotech Co., Ltd, we evaluated the performance of a cTnT quantitation kit, a chemiluminescence microparticle immunoassay, using a CARIS series chemiluminescence instrument developed by Xiamen Excellent Maike Medical Instrument Co, Ltd. Performance evaluation of the product included the following aspects: detection limit, linear and reportable ranges, accuracy, precision, resistance to interference, cross reactivity, matrix effect, and etc. Meanwhile, the performance of the product was compared with an electrochemiluminescence-based cTnT quantitation kit (Roche, Basel, Switzerland).

2 | MATERIALS AND METHODS

2.1 | Sample source

All serum samples were collected from the clinical test for cTnT target abnormalities, cTnT indicators of normal, other indicators of myocardial markers (cTnI) abnormal patients and healthy samples in Xiamen University Affiliated Zhongshan Hospital, Xiamen, China, in July 2013. The serum samples were separated by centrifugation (800×*g*, 10 minutes) and stored at −80°C until used. Informed consents were obtained from all individual participants.

2.2 | Instruments

CARIS series chemiluminescence instrumentation (Xiamen Excellent Maike Medical Instrument Co., Ltd, Xiamen, China) and ODULAR Analytics E170 (Roche, Basel, Switzerland).

2.3 | Reagents

The quantitation kit for cTnT detection produced by Xiamen InnoDx Biotech Co., Ltd. was employed. Another chemiluminescence cTnT quantitation kit developed by Roche served as a gold standard. The interference factors used in this study included hemoglobin (HyTest), bilirubin (Aladdin Chemistry), triglycerides (Aladdin Chemistry), rheumatoid factor (Biorbyt), skeletal troponin I (HyTest), cardiac troponin I (International laboratory, USA), human troponin C (HyTest), and skeletal troponin T (HyTest). L-series standard solutions as well as blank reference and working calibrators were obtained from Xiamen InnoDx Biotech Co, Ltd.

2.4 | Evaluation of detection limits

L-series standard solutions (L1-L8, with concentrations of 0.01 ng/mL, 0.1 ng/mL, 0.4 ng/mL, 1 ng/mL, 2 ng/mL, 5 ng/mL, 10 ng/mL, and 25 ng/mL, respectively) were simultaneously measured, employing the product being tested. Dose-response curves were obtained by fitting results with a four-parameter logistic curve (4PLC). Meanwhile, the blank reference was tested repeatedly 20 times, then, the average of the results was put into the dose-response curve, to achieve the detection limits.

2.5 | Evaluation of linear range

Following the EP6-A protocol, an approved guideline published by the Clinical and Laboratory Standards Institute (CLSI, USA),[7] and according to "Guiding principles on performance analysis of diagnostic reagents in vitro", which was formulated by the Review Center of the State Food and Drug Administration Medical Device Technology (CMDE) in China and mainly referenced to the Clinical and Laboratory Standards Institute (CLSI) documents. The concentrations of L-Series standard solutions (L1-L8 at known concentration of 0.01 ng/mL, 0.1 ng/mL, 0.4 ng/mL, 1 ng/mL, 2 ng/mL, 5 ng/mL, 10 ng/mL, and 25 ng/mL, respectively) were measured by the test product. Multiple regression was applied, with data plotted using the known concentration of samples on an *X*-axis and the measured average values on the *Y*-axis, with both axes plotted on a base 10 logarithmic scale. The linear range of the test product was evaluated by the average deviation from linearity (ADL) of the obtained first-, second-, and third-order polynomials.

2.6 | Evaluation of reportable range

Following the EP6-A guideline published by CLSI and in accordance with the "Guiding principles on performance analysis of diagnostic reagents in vitro", the test product was used to repeatedly detect low-value serum samples with five concentrations (Y1, Y2, Y3, Y4, and Y5) and high-value serum samples with three different concentrations (H1, H2, and H3). The lower limit of the reportable range was defined as the lowest measured concentration at which the coefficient of variation (CV) equaled to, or was smaller than, 10% of the measured concentration. The higher limit of the reportable range was defined as the recovered concentration of the high-value serum samples, when the recovered concentration differed by less than 10% of the theoretical concentration.

2.7 | Accuracy evaluation

The recovery rate is an important indicator of the testing accuracy of reagent quantitation. This procedure followed the EP9-A2 guideline published by CLSI[8] and was in accordance with the "Guiding principles

on performance analysis of diagnostic reagents in vitro". The accurately calibrated master calibrator was first diluted to a concentration within the detection range of the kit. Meanwhile, a normal human serum sample was obtained. Twenty microliter of the diluted calibrator was mixed with 180 μL of the normal human serum sample, producing a mixed sample. Repeat three times to detect three batches and calculate the recovery rate.

2.8 | Precision evaluation

Precision is an indicator of the degree of random error in a measurement. It is an important index for quantitative experiments. The smaller the CV, the better the repeatability. Following the EP5-A guideline published by CLSI,[9] test products from three different batches were used to measure cTnT concentrations of serum samples 1 and 2. Each sample was tested in parallel 20 times to calculate the mean and standard deviation (SD) from the measured values. In addition, coefficients of variance within and in between batches were calculated.

2.9 | Cross-reaction verification

To examine whether the reagent cross-reacted with other compounds, the test product was used to detect negative serum samples containing other compounds, including skeletal troponin I (500 ng/mL), cardiac troponin I (500 ng/mL), human troponin C (500 ng/mL), and skeletal troponin T (500 ng/mL).

2.10 | Interference test

This procedure followed the EP7-A2 guideline published by CLSI[10] and was in accordance with the "Guiding principles on performance analysis of diagnostic reagents in vitro". The test product was used to examine hemoglobin-, bilirubin-, triglyceride-, and rheumatoid factor-containing solutions that were diluted in a control serum sample. Interference effects of hemoglobin, bilirubin, triglycerides, and rheumatoid factor on the accuracy of test product were evaluated. Interference was considered significant when the percent bias (Bias%), calculated from the percent difference before and after addition of interference factors, was greater than ±10%.

2.11 | Evaluation of matrix effect

The matrix effect of a tested serum sample was assessed according to the EP14-A2 guideline published by CLSI.[11] A high-value serum sample was separately diluted into gradient with two different dilution buffers, a sample dilution solution and a matrix serum. The concentrations were measured by the test product from the same batch. Results were visualized on a scatter plot with the known concentration of samples along the *X*-axis and the measured results along the *Y*-axis. The matrix effect of the serum sample was assessed by comparing the scatter distribution of samples prepared by two different methods.

2.12 | Correlation study

The test and reference products were used in parallel to measure the cTnT concentrations of serum samples from 220 patients. Measurements were carried out on a CARIS series chemiluminescence instrument (Xiamen Excellent Maike Medical Instrument Co., Ltd) and MODULAR Analytics E170 (Roche, Basel, Switzerland), respectively. A scatter plot was generated with measured results from the reference product plotted on the X-axis, and results from the test product plotted on the Y-axis. The degree of correlation was quantified from the linear regression equation of the plot. Based on the results, patients were grouped into cTnT normal group or cTnT abnormal group; a cTnT concentration of <0.1 ng/mL was considered normal. The consistency of two products was compared by calculating the overall, normal group, and abnormal group matching rates.

2.13 | Statistical analysis

Statistical analysis was performed using Microsoft Office Excel 2016 and SPSS 19.0 software. The ADL was calculated from a multiple regression analysis. The nonlinearity of polynomials was considered clinically acceptable when the ADL was less than a corresponding threshold. The degree of consistency between two products was evaluated by the Interrater Reliability (Kappa) test and the consistency was statistically significant when $P < .05$. The degree of correlation was evaluated by Pearson's *R* Correlation test and the correlation between two sets of data was considered statistically significant when $P < .05$.

3 | RESULTS

3.1 | Evaluation of detection limit

The test product was used to measure the concentrations of diluted gradient L-series standards and to generate standard dose-response curves (Figure 1). Meanwhile, the cTnT concentration of the 0 ng/mL standard solution (blank reference) was measured 20 times in detection independently. The average values of the lowest detected concentrations were 0.0092 ng/mL (Table 1); below 0.01 ng/mL. Therefore, the lowest detection limit of the test product was 0.01 ng/mL.

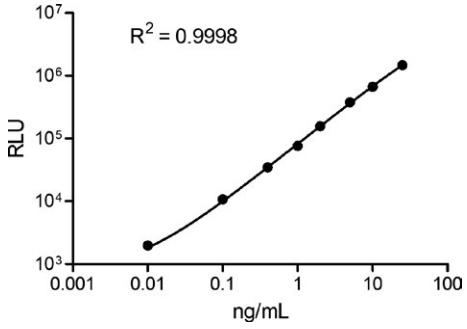

FIGURE 1 The standard dose-response curves of the kit

TABLE 1 Determination of the limit of detection

Mean of RLU	SD	LOD (ng/mL)
1254	248	0.0092

RLU, relative light units; SD, standard deviation; LOD, limit of detection.

3.2 | Evaluation of linear range

The polynomial obtained from regression analysis was used for a linearity test with a clinical standard. When the confidence interval was set at 90%, the ADL of both second- and third-order regression equations was less than the threshold, with clinically acceptable nonlinearity. Therefore, the linear range of this test product was 0.01 ng/mL -25 ng/mL.

3.3 | Evaluation of the reportable range

The results of the reportable range are shown in Tables 2 and 3. The CV values were all within the acceptable limits (10%) for low-value serum samples at 0.008-0.012 ng/mL cTnT. Therefore, cTnT at the concentration of 0.01 ng/mL was chosen as the lower limit of the reportable range. The percent of bias between the recovered and theoretical concentrations was always under 10% when high-value serum samples were diluted by two- to fourfold. A fourfold dilution was therefore recommended as the maximum dilution factor. As the upper limit of a kit's reportable range is determined as the product of the upper limit of its linear range and its maximum dilution factor, the upper limit of the reportable range for the test product was 100 ng/mL.

3.4 | Accuracy evaluation

The recovery rate was determined to be between 93.5% and 102.0%; the quantitation accuracy of the product was therefore good.

3.5 | Precision evaluation

As shown in Table 4, the test products from three different batches have an intrabatch CV of 2.9%-6.4% and an interbatch CV of 6.0%, suggesting that results of testing kit has a good repeatability.

3.6 | Cross-reaction validation

The test product demonstrated no cross reactivity with skeletal troponin I, cardiac troponin I, human troponin C, or skeletal troponin T, as summarized in Table 5.

TABLE 5 Validation of the cross reaction of the kit

Compound	Concentration	Detection of cTnT
Skeletal troponin I	500 ng/mL	Negative
Cardiac troponin I	500 ng/mL	Negative
Human troponin C	500 ng/mL	Negative
Skeletal troponin T	500 ng/mL	Negative

TABLE 2 Lower limit of reportable range

	L (ng/mL)	L2 (ng/mL)	L3 (ng/mL)	L4 (ng/mL)	L5 (ng/mL)
Mean±SD	0.0074 ± 0.001	0.0083 ± 0.001	0.0095 ± 0.001	0.0105 ± 0.001	0.0116 ± 0.001
CV%	10%	9%	8%	7%	6%

TABLE 3 Upper limit of reportable range

	H1 (ng/mL)	H2 (ng/mL)	H3 (ng/mL)
Mean±SD	13.6930 ± 1.0216	14.5747 ± 0.7576	14.8038 ± 1.6957
Diluent fold	2	4	6
Restore concentration	27.3859	58.2987	88.8226
Theoretical concentration	27	54	81
Bias (%)	1%	8%	10%

TABLE 4 Evaluation of intra- and interbatches precision

	Low level			High level		
Lot number	Mean (ng/mL)	Range (ng/mL)	Intra-batch CV	Mean (ng/mL)	Range (ng/mL)	Intrabatch CV
A	1.0462	0.9733-1.1352	5.0%	10.3811	9.4745-11.3076	6.4%
B	0.9875	0.8983-1.0650	5.8%	9.6518	9.2244-9.9506	2.9%
C	0.9946	0.8976-1.1132	6.0%	10.1647	9.3498-11.2366	5.7%
Interbatch CV	6.0%			6.0%		

3.7 | Interference testing

As shown in Table 6, when interfering substances (hemoglobin at a concentration of under 1000 μg/mL, bilirubin at under 250 μg/mL, or rheumatoid factor at under 206 U/mL) were added, test results were not affected; the percent bias (Bias%) was within ±10% for each variable. Triglycerides at a concentration of 3.75-11.25 mg/mL had little impact on test results as well.

3.8 | Evaluation of matrix effects

Diluting samples with a sample dilution buffer or matrix serum had no statistically significant difference on the detection range ($P = .091 > .05$).

TABLE 6 Interference testing of the kit

	Result	
Interference	**cTnT (ng/mL)**	**Bias (%)**
Hemoglobin (μg/mL)		
250	0.0693	0.14
500	0.0656	−5.20
750	0.0718	3.76
1000	0.0695	0.43
Bilirubin (μg/mL)		
60	0.0654	5.65
130	0.0605	−2.26
190	0.0634	2.42
250	0.0601	−2.91
Triglyceride (mg/mL)		
3.75	0.0797	−4.09
7.50	0.0793	−4.57
11.25	0.0768	−7.58
15.00	0.0886	6.62
Rheumatoid factor (U/mL)		
51.5	0.0653	8.47
103.0	0.0610	1.33
154.5	0.0625	3.82
206.0	0.0646	7.31

3.9 | Correlation study

cTnT concentrations in serum samples from 220 patients were measured by both test and reference products in parallel, with results subjected to a linear regression analysis. A linear regression equation was obtained: $Y = 1.027X–0.1829$ ($R^2 = 0.9325$, $P < .001$) (Figure 2A). Based on Bland-Altman analysis, the average difference between the two methods was –0.135464, and the limits of agreement were 0.288423 to –0.559352 (Figure 2B). In addition, the qualitative results obtained from test and reference products were both highly consistent (Kappa = 0.900, $P < .001$) and correlated ($r = .903$, $P < .001$). The matching rate for the abnormal group reached 90.83% (diagnostic reference value is 100 pg/mL), while for the control group it reached 99.01%. The overall matching rate was 95.00%.

4 | DISCUSSION

cTnT is an important marker for heart failure and a vital indicator for both the diagnosis and prognosis of acute myocardial infarction. Increased levels of troponins are associated with severity and poorer prognosis of coronary artery disease (independent of natriuretic peptide BNP or NT-proBNP). Increased cTnT concentration in the blood is also seen in other diseases such as myocarditis, cardiac contusion, pulmonary embolism, and drug-induced cardiotoxicity.

At present, there is a lack of independent, domestic development of an in vitro cTnT diagnostic kit in China. Domestically, cTnT levels are mostly evaluated with an expensive electrochemiluminescence-based kit developed by Roche. With the increasingly fierce competition in the field of detection using in vitro diagnostic kits in China, imported reagents dominate the market. It is therefore imperative and socially meaningful to develop and promote domestically produced cTnT chemiluminescence immunoassay kits with satisfactory performance that can effectively reduce medical expenses and lighten the financial burden of patients.

Xiamen InnoDx Biotech Co., Ltd. and our laboratory jointly evaluated the performance of a chemiluminescence microparticle immunoquantitation kit for cTnT detection, according to instructions from the "Guiding principles on performance analysis of diagnostic reagents in vitro". CLSI was referred to for relevant standard procedures. Performance parameters of the kit included sensitivity, linear and reportable ranges,

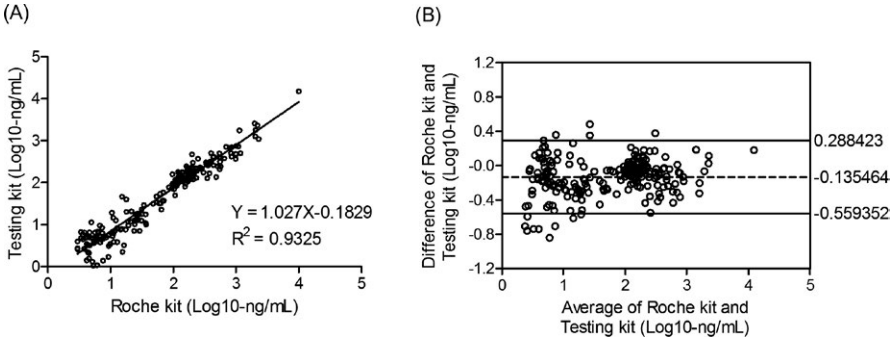

FIGURE 2 (A) Linear regression analysis of the detection of cTnT between test and reference products; (B) Bland-Altman analysis for 220 serum samples detected by test and reference products

accuracy, precision, cross reactivity, and interference resistance. The test product was also compared with an electrochemiluminescence-based cTnT quantitation kit developed by Roche. Results suggested that all tested performance parameters of the kit produced by Xiamen InnoDx Biotech Co., Ltd. met clinical requirements.

The limit of detection validated by the kit (0.01 ng/mL) no better than the one assigned by Roche (0.003 ng/mL). Only minimal deviations were observed in the test of reportable range, indicating that assay measurement was reliable across the range of 0.01 ng/mL-25 ng/mL. The upper limit of 100 ng/mL was higher than 10 ng/mL, in comparison to reportable range of Roche. In this study, we evaluated the degree of potential interference of abnormal clinical samples that could result from hemolysis, jaundice, hyperlipemic blood, or rheumatoid factor by adding hemoglobin, bilirubin, triglycerides, and rheumatoid factor to tested solutions, respectively. As |Bias|≤10% was set as a medical reference level for interference tests, the deviation of test results remained acceptable for hemoglobin levels under 1000 μg/mL, bilirubin under 250 μg/mL, triglycerides under 11.25 mg/mL, and rheumatoid factor under 206 U/mL. In addition, the above-mentioned interference factors within the indicated concentration ranges did not interfere with the cTnT detection. However, the interference from these factors at a higher concentration on cTnT detection remains to be verified. Based on the interference test results, the manufacturer was suggested to further improve the quality of test products to enhance interference resistance.

Cross-reactivity evaluation revealed that the test product did not provide false-positive readings when incubated with troponin subtypes such as skeletal troponin I, cardiac troponin I, human troponin C, and skeletal troponin T. The high specificity of cTnT plays an important role in clinical differential diagnosis. For examples, cTnT could serve as a marker for patients with myocardial damage in association with skeletal muscle injury.[12] Matrix-effect assessment revealed that there was no matrix effect for samples in serum.

During correlation study of the tested and reference products, 220 serum samples were analyzed by both products. The obtained results were subjected to a quantitative correlation analysis and a qualitative matching rate analysis. These analyses suggested that results obtained by both products were highly correlated. The test product was accurate enough to distinguish whether cTnT was abnormal in clinical samples.

In summary, the general performance of the cTnT chemiluminescence quantitation kit developed by Xiamen InnoDx Biotech Co., Ltd. based on CARIS series instrument is acceptable. This cTnT quantitation kit meets requirements for application in clinical settings and is more financially advantageous than reference products.

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