

## RESEARCH ARTICLE

# Evaluation of a newly developed quantitative determination kit for tumor marker CA15-3 with chemiluminescent assay

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**Background:** Tumor marker carbohydrate antigen 15-3 (CA15-3) is used as a biomarker to aid to diagnose and monitor the prognosis of breast cancer patients. A new quantitative determination kit for CA15-3 with chemiluminescent assay was developed by Xiamen InnoDx Biotech Co., Ltd, China. Therefore, we conducted the report to evaluate the performance of the kit.

**Methods:** According to the "Guiding principles on performance analysis of diagnostic reagents in vitro", the calibration curve, limit of detection, reportable range, accuracy, precision, anti-interference capability, cross-reaction and comparison by measuring EDTA plasma and serum were carried out. In addition, the kit was performed in parallel to electrochemiluminescence immunoassay kit (Roche) to analyze the correlation between the two kits.

**Results:** Regression equation of calibration curve of the kit was  $Y=0.7914X+4.1032$  ( $R^2=.990$ ). Limit of detection was 0.0347 U/mL. The reportable range was 0.5-2400 U/mL. Recovery ratio was 100.0%-104.8%. Coefficient of variations (CVs) of within-run and between-run were 4.8%-7.6% and 5.8%-7.4% respectively. No remarkable interferences (all Bias% were less than  $\pm 10\%$ ) were detected when samples contained hemoglobin  $\leq 183.8 \mu\text{mol/L}$ , bilirubin  $\leq 340 \mu\text{mol/L}$ , triglyceride  $\leq 18.1 \text{mmol/L}$ , or rheumatoid factor  $\leq 400 \text{U/mL}$ . No cross-reaction was present in the kit. Moreover, compared with the results from electrochemiluminescence immunoassay kit (Roche) in 345 serum samples, there was a satisfied correlation coefficient of 0.977 ( $P<.01$ ), and the kit was simultaneously fit for the detection of EDTA plasma and serum samples.

**Conclusion:** The new kit validated satisfactorily, and it can be used for detecting CA15-3 in clinical practice.

## KEYWORDS

carbohydrate antigen 15-3, chemiluminescent, evaluation, kit, performance

## 1 | INTRODUCTION

Breast cancer is the first most commonly diagnosed cancer and second leading cause of cancer related death in women in the USA.<sup>1</sup> Some examinations are performed for diagnosing and monitoring the prognosis

of patients with breast cancer in clinical practice. Along with the traditional prognostic elements such as tumor size, tumor grade, lymph node status, molecular markers including hormone receptor status and human epidermal growth factor receptor 2 (HER2) expression,<sup>2</sup> serum tumor markers play an important role in screening, early diagnosis of

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recurrence, and treatment of many malignancies.<sup>3,4</sup> CA15-3, a member of the mucin-1 (MUC-1) family of glycoproteins, was one of the soluble molecules released into the blood stream by breast cancer cells or other cell types belonging to tumor microenvironment acts as a serum tumor marker.<sup>5,6</sup> It was found as the first breast cancer-associated antigen in 1984.<sup>7</sup> After decades of experimental research on it, investigators had found that CA15-3 could be used as diagnostic and prognostic factor and could also provide valuable information during follow-up.<sup>8-11</sup> Thus, measurement of CA15-3 is significant for breast cancer patients.

Quantitative determination kits for CA15-3 with chemiluminescent assay produced by Beckman and Abbott, and Elecsys kit provided by Roche (Basel, Switzerland) are the main reagents for CA15-3 clinical measurement in China. Nowadays, Chinese market for *in vitro* diagnostic assay for CA15-3 is mainly occupied by the above three high cost foreign reagents, especially by the Roche. For breaking the monopoly phenomenon, reducing the cost of testing, and allaying the financial burden of patients, a new chemiluminescent assay kit for CA15-3 with low cost and yet effective measurement was developed by Xiamen InnoDx Biotech Co., Ltd., Xiamen, China.

How about the performance validation of the new chemiluminescent assay kit for CA15-3? Thus, Xiamen InnoDx Biotech Co., Ltd cooperated with our lab to evaluate the kit from the aspects including the calibration curve, limit of detection, reportable range, accuracy, precision, anti-interference capability, cross-reaction, measurement comparison between plasma and serum samples, and method comparison with Roche kit.

## 2 | MATERIALS AND METHODS

### 2.1 | Sample collection

All the samples analyzed in the study were collected from the patients with breast cancer, benign breast disease or other cancers, and the healthy people on quantitation of CA15-3 in Xiamen University Affiliated Zhongshan Hospital, Xiamen, China in July 2013. The serum or EDTA plasma samples were separated by centrifugation (800 g, 10 minutes) and stored at  $-30^{\circ}\text{C}$  until used.

### 2.2 | Apparatus

The chemiluminescence apparatus of CARIS (Xiamen excellent Maiké Medical Instrument Co., Ltd, Xiamen, China) and COBASe601 (Roche) were utilized in this study.

### 2.3 | Chemicals and reagents

(i) Testing kit: the quantitative determination kit for CA15-3 with chemiluminescent assay, which developed by Xiamen InnoDx Biotech Co., Ltd. (ii) Comparative kit: the quantitative determination kit for CA15-3 with electrochemiluminescent immunoassay, procured from Roche. (iii) Interferential substances: hemoglobin; bilirubin; triglyceride; rheumatoid factor. (iv) Cross-reaction substances: carbohydrate antigen 125 (CA125), carbohydrate antigen 19-9 (CA19-9), carbohydrate antigen 242 (CA242), alpha-fetoprotein (AFP), prostate specific antigen (PSA) and cytokeratin-19 soluble fragment (CYFRA 21-1).

## 2.4 | Performance validation

Performance of the calibration curve, limit of detection, reportable range, accuracy, precision, anti-interference capability, cross-reaction, method comparison between Roche kit and testing kit, and comparison by measuring plasma and serum were validated according to "Guiding principles on performance analysis of diagnostic reagents *in vitro*", which 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. Specifically, experiments included calibration curve according to CLSI EP6-A,<sup>12</sup> precision according to CLSI EP5-A,<sup>13</sup> anti-interference capability according to CLSIEP7-A,<sup>14</sup> and method comparison according to CLSIEP9-A2.<sup>15</sup>

### 2.4.1 | Calibration curve

Calibration curve was generated to confirm the linear relationship between the detection results of relative light units (RLU) and the concentration of calibration solutions. The calibration solutions were at the concentrations of 0.025, 0.1, 0.5, 2, 20, 100, 300, 900 U/mL respectively. Specially, a logarithmic transformation was applied to the titers to obtain a normal distribution of the data. The log of RLU and the log of matched concentrations of calibration solutions were utilized as *y* and *x* variables in a standard regression analysis to evaluate the linearity.

### 2.4.2 | The limit of detection

The mean and standard deviation (SD) of the calibration solution at the level of 0.0 U/mL were determined by 20 consecutive measurements. Subsequently, calculating from the mean+2SD of RLU, based on calibration curve, to achieve the actual concentration correspondingly, which was the limit of detection.

### 2.4.3 | Reportable range

Reportable range was analyzed by measuring ten replicates of the serum samples, which were at two different levels. Among them, four serum samples at low level were recorded as L1, L2, L3, L4, and three-ones at high level were recorded as H1, H2, H3. To extend the reportable range beyond the upper limit of the kit, 10 $\times$  dilutions were evaluated in serum sample at high level. Reportable range was determined by the measurement, of which the percent of CVs and relative bias% were within  $\pm 10\%$ .

### 2.4.4 | Accuracy

Assess accuracy by the recovery study. In the recovery study, three solutions were prepared. The first solution was the dilution of calibration solution, the second one was the serum sample of healthy people, and the third one was achieved as follows: 20  $\mu\text{L}$  of first

solution was spiked with 180  $\mu\text{L}$  of second one to obtain the mixture. Then, the accuracy was evaluated by measuring the recovery rate.

### 2.4.5 | Precision

Precision was evaluated by calculating the coefficients of variations (CVs) of within-run and between-run. The experiments were performed using two concentrations of serum samples, which were analyzed twenty times over three lots (lot A, lot B and lot C) respectively.

### 2.4.6 | Anti-interference capability

To identify if increased concentrations of commonly occurring sample matrix components would interfere with the accuracy of the kit assay, the effect of elevated hemoglobin, bilirubin, triglyceride and rheumatoid factor was evaluated using additional interferences. Anti-interference capability was analyzed by calculating the relative bias%.

### 2.4.7 | Cross reaction

Serum samples with CA15-3 <25 U/mL (recorded as negative) were utilized in the experiments to determine the present of cross-reaction in the kit. CA125(5000 U/mL), CA19-9(1000 U/mL), CA242(200 U/mL), AFP(1000 IU/mL), PSA(100 ng/mL) and CY21-1(1000 ng/mL) were spiked individually into the serum samples. All the substances were assayed and CA15-3 of samples was also detected again.

### 2.4.8 | Method comparison

Comparison of methods from different manufacturers was also carried out. The level of serum tumor marker CA 15-3 was parallelly evaluated by the kit and Roche kit in our present study including 345 patients, to analyze the correlation between the two kits. The log of measurement of CA15-3 was utilized.

### 2.4.9 | Plasma and serum

CA15-3 measurement in EDTA plasma and serum was compared by testing 150 matched pairs of EDTA plasma and serum samples with dose values covering the entire reportable range of the assay, and the difference of the results were assayed. The log of detection of CA15-3 was analyzed.

## 2.5 | Statistic analysis

All statistics were completed using the SPSS software (SPSS version 20.0, Chicago, IL, USA) and GraphPad Prism (GraphPad Software, La Jolla, CA, USA). Pearson contingency coefficient was conducted to expound correlation between the testing kit and Roche kit, and the

equation was generated by simple linear regression analysis.  $P < .05$  was considered statistically significant.

## 3 | RESULTS

### 3.1 | Calibration curve

The represented calibration curve of the analytes was shown in Figure 1, which presented good linearity. The typical regression equation was  $Y = 0.7914X + 4.1032$  ( $R^2 = .990$ ).

### 3.2 | The limit of detection

The limit of detection was 0.0347 U/mL (Table 1).

### 3.3 | Reportable range

The results were listed in Tables 2 and 3, all the CVs% and bias% were all within  $\pm 10\%$ . The lower limit of reportable range was directly given by the test concentration of the serum sample. The  $10\times$  on-board dilution extended the upper end of the reportable range to 2400 U/mL. Consequently, the reportable range was 0.5-2400 U/mL.

### 3.4 | Accuracy

The mean recovery percentage ranged from 100.0% to 104.8%, which indicated an acceptable degree of accuracy by the kit.

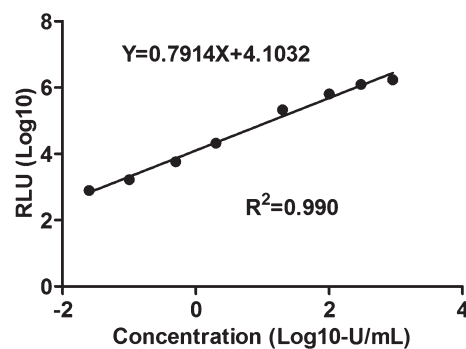


FIGURE 1 The calibration curve of the kit

TABLE 1 Determination of the limit of detection

Mean of RLU	SD	LOD (U/mL)
719	84.2	0.0347

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

TABLE 2 Lower limit of reportable range

	L1 (U/mL)	L2 (U/mL)	L3 (U/mL)	L4 (U/mL)
X $\pm$ S	0.39 $\pm$ 0.01	0.48 $\pm$ 0.02	0.27 $\pm$ 0.02	0.64 $\pm$ 0.05
CV%	2.5%	3.1%	8.8%	7.2%

Four serum samples at low level: L1, L2, L3, L4.

**TABLE 3** Upper limit of reportable range

	H1 (U/mL)	H2 (U/mL)	H3 (U/mL)
X±S	158.24±1.82	190.90±12.70	256.93±20.79
CV%	1.15%	6.65%	8.09%
Diluent fold	2	5	10
Restore concentration	316.48	954.50	2569.33
Theoretical concentration	300	900	2400
Bias (%)	5.5%	6.1%	7.1%

Three serum samples at high level: H1, H2, H3.

### 3.5 | Precision

The precision results were summarized in Table 4. The CV of within-run precision was 4.8%-7.6%, and the CV of between-run precision was 5.8%-7.4%, <10%, which demonstrated a satisfactory repeatability.

### 3.6 | Anti-interference capability

Analysis of interfering substances revealed that the bias % were all within ±10%, that was, no statistically significant difference between serum samples without interferences and samples containing hemoglobin at concentrations up to 183.8 µmol/L, bilirubin at concentrations up to 340 µmol/L, triglyceride at concentrations up to 18.1 mmol/L, or rheumatoid factor at concentrations up to 400 U/mL (Table 5).

### 3.7 | Cross reaction

CA15-3 in serum samples, which mixed the additional substances, was less than the concentration of 25 U/mL (recorded as negative). The results were listed in Table 6, which suggested that no cross-reaction existed between the kit and the other tumor markers, such as CA125, CA19-9, CA242, AFP, PSA and CY21-1.

### 3.8 | Method comparison

A satisfactory relevance and consistency were observed. The correlation study with the kit and Roche kit demonstrated a similarity between the two methods ( $r=0.977$ ,  $P<0.01$ ). The regression equation of the two methods response was  $Y=0.993X-0.001$  ( $R^2=0.954$ ,  $P<0.01$ )

**TABLE 5** Evaluation of anti-interference capability

Interference	Result	
	CA15-3 (U/mL)	Bias (%)
Hemoglobin (µmol/L)		
0	12.07	—
36.8	12.62	4.56
73.5	12.19	0.99
110.3	11.95	-0.99
147.1	13.23	9.61
183.8	12.39	2.65
Bilirubin (µmol/L)		
0	12.67	—
34	12.49	1.42
85	12.80	1.03
170	13.67	7.89
340	13.24	4.50
Triglyceride (mmol/L)		
0	13.11	—
2.3	13.78	5.11
4.5	13.00	-0.84
9.0	13.95	6.41
18.1	13.57	3.51
Rheumatoid factor (U/mL)		
0	12.42	—
50	13.08	5.31
100	11.41	-8.13
200	13.45	8.29
400	12.98	4.51

(Figure 2A). On the basis of Bland-Altman analysis, the mean difference between the two methods was -0.0094, and the limits of agreement were -0.18 to 0.16 (Figure 2C).

### 3.9 | Plasma and serum

The results indicated a good correlation between the measurement of EDTA plasma and serum samples ( $r=0.938$ ,  $P<0.01$ ), and the regression equation obtained was  $Y=0.962X+0.033$  ( $R^2=0.878$ ,  $P<0.01$ ) (Figure 2B). Bland-Altman analysis demonstrated that the mean difference

**TABLE 4** Evaluation of within- and between-runs precision

Lot number	Low level			High level		
	Max (U/mL)	Min (U/mL)	Within-run CV	Max (U/mL)	Min (U/mL)	Within-run CV
A	2.19	1.83	5.9%	110.72	88.29	7.3%
B	2.24	1.89	4.8%	110.76	86.94	7.2%
C	2.23	1.83	6.8%	114.93	88.07	7.6%
Between-run CV	5.8%			7.4%		

**TABLE 6** Evaluation of the cross-reaction of the kit

Compound	Concentration	Detection of CA15-3
A125	5000 U/mL	Negative
CA19-9	1000 U/mL	Negative
CA242	200 U/mL	Negative
AFP	1000 IU/mL	Negative
PSA	100 ng/mL	Negative
CY21-1	1000 ng/mL	Negative

Negative: the concentration of CA15-3 was <25 U/mL.

between the EDTA plasma and serum samples was  $-0.0063$  and that the limits of agreement were  $-0.20$  to  $0.18$ , which demonstrated the satisfactory consistency between the measurement of EDTA plasma and serum samples (Figure 2D).

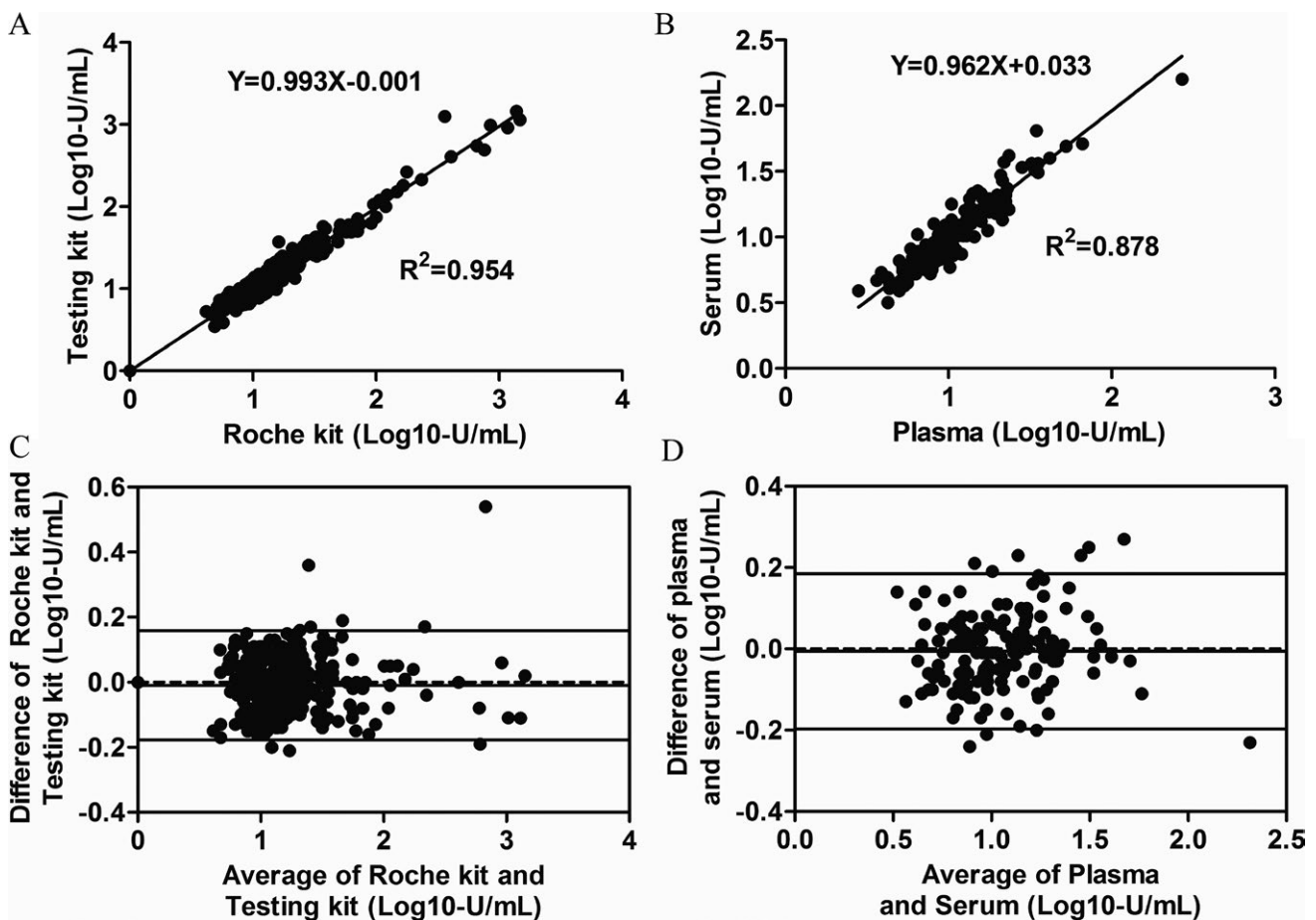
## 4 | DISCUSSION

CA15-3 is the tumor-associated biomarker that has been popularly utilized in screening, identification, prognosis or detection breast

cancer and it has been approved by the Food and Drug Administration (FDA) as a marker to monitor chemotherapy in advanced breast cancer patients.<sup>16</sup> Optimal care of patients with breast cancer needs to measure CA15-3 and interpret the concentrations in conjunction with other clinical information and laboratory data.

Currently, available quantitative determination kits for CA15-3 measuring in China are mainly purchased from Beckman (chemiluminescent assay kit), Abbott (chemiluminescent assay kit) and Roche (electrochemiluminescence immunoassay kit). Patient testing using these methods requires a high cost because kits above got abroad. The strategy of breaking the foreign monopoly, reducing the cost of measurement encourages the replacement of the foreign kits. In this context, a new quantitative determination kit for CA15-3 with chemiluminescent assay has been developed by Xiamen InnoDx Biotech Co., Ltd, China. Subsequently, the full validation study of the kit was performed by Xiamen InnoDx Biotech Co., Ltd and our laboratory.

The results of calibration curve indicated a good linearity at the concentration ranging from  $0.025$  to  $900$  U/mL. The limit of detection validated by the kit ( $0.0347$  U/mL) being lower than the one assigned by Roche ( $1.00$  U/mL). Only minimal deviations were observed in the test of reportable range, indicating that assay measurement



**FIGURE 2** Linear regression analysis of the detection of CA15-3 between (A) the Roche kit and the testing kit; (B) the EDTA plasma and the serum samples. Bland-Altman analysis for (C) 345 serum samples detected by Roche kit and the testing kit; (D) 150 EDTA plasma and the serum samples measured by testing kit. The determined bias is  $-0.0094$ (Log10-U/mL) and  $-0.0063$ (Log10-U/mL) respectively

was reliable across the range of 0.5–2400 U/mL. The upper limit of 2400 U/mL was lower than 3000 U/mL, in comparison to reportable range of Roche. Only 10× dilutions were evaluated in this trial, maybe higher fold of dilutions are demanded to further validate the upper limit of reportable range. In addition, the accuracy was satisfactory because of the percentage of recovery ranging from 100.0% to 104.8%, and the precision results revealed that the test performs well, as evidenced by the low within-run and between-run CVs, which were all within 10%. Interference due to hemoglobin, triglyceride, bilirubin and rheumatoid factor was within ±10% of the reference CA15-3 measurement when the interferents were spiked with at a certain concentrations. No cross-reactions were detected between the testing kit and the substances of CA125, CA19-9, CA242, AFP, PSA and CY21-1. Moreover, CA15-3 quantitation by Roche kit was carried out in parallel to the kit, a strong correlation was observed between the two kits, which reflected in the slope of 0.993 and a correlation of  $r=0.954$  and  $P<0.01$ , and the satisfactory results of Bland-Altman analysis. It was also necessary to determine if it was valid to combine results from serum and EDTA plasma samples. A comparison study of paired serum and EDTA plasma samples was conducted, both serum and EDTA plasma can be used as sample type in the assay as their linear regression analysis showed a slope of 0.962 and a correlation of  $r=0.938$  and  $P<0.01$ , and the analysis of Bland-Altman displayed a great consistency.

According to the results, the quantitative determination kit for CA15-3 with chemiluminescent assay developed by Xiamen InnoDx Biotech Co., Ltd, China has the value of application for clinical measurement, and has shown its potential competitiveness with less cost and yet effective measurement.

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## AUTHOR CONTRIBUTIONS

ZY Zhang and FH Xu conceived experiments. PH Li and ZY Zhang wrote the manuscript. PH Li, HM Ye, JW Liu and HW Jin prepared tables and figures. HM Ye, YZ Lin, YY, SD Yan and LG performed experiments. All authors discussed the results and commented on the manuscript.

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