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RESEARCH ARTICLE

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Comparison of seven different reagents of peroxidase method for small and dense low density lipoprotein cholesterol (sdLDL-C) measurement

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Abstract

Background: We validated the performance of seven different reagents of peroxidase method for sdLDL-C in two automatic analyzers that are common in Chinese laboratories.

Methods: Seven commercially available sdLDL-C assays were analyzed with the Beckman AU5400 and Mindray BS2000 automatic analyzers. A total of 336 blood samples were collected and the reference interval was also validated in 298 apparently healthy individuals. Serum samples were used for method comparison of precision, recovery, lower limit of detection, comparison and concurrence analysis, as well as reference interval for the Mindray reagent.

Results: The repeatability CV% of the seven sdLDL-C assays were 0.81%~3.66% for Mindray BS2000 and 0.76%~3.91% for Beckman AU5400, while Total CVs for Mindray BS2000 sdLDL-C assay were 1.34%~4.81%, and that of Beckman AU5400 were 2.25%~10.33%. The measured recovery rates of sdLDL-C assays were within the allowable ±10% deviation range. There was no obvious difference between the reagents in the lower limit detection. There was a difference between the validation results of the reference range and the manufacturer's.BSBE, Mindray, and Dongou had a high degree of association with DENKA SEIKEN on Mindray BS2000, while BSBE, Mindray, Dongou and Merit Choice had a high degree of association with DENKA SEIKEN on Beckman AU5400. Passing–Bablok regression showed excellent linear correlation between BSBE and Mindray and DENKA SEIKEN and on Beckman AU5400.

Conclusions: Our results indicate that the basic performance can meet the testing requirements, but the comparability between them is still insufficient.

KEYWORDS
method comparison, peroxidase method, reference interval, small and dense low density lipoprotein cholesterol

Fan Xuesong and Wang Enshi contributed equally on this work.

1 | INTRODUCTION

Low-density lipoprotein cholesterol (LDL-C) is heterogeneous and consists of a series of particles with different size, density and chemical composition. LDL-C as a traditional risk factor of atherosclerosis, has been shown to be closely related to cardiovascular and cerebrovascular disease.[1] Many guidelines recommend LDL-C as a lipid-lowering target.[2] However, it can be seen that many patients still have acute cardiovascular and cerebrovascular events even though LDL-C is in the normal range, and many patients still have the disease even though they use statins to reduce LDL-C to the normal range, so it is insufficient to evaluate the risk of these patients only with LDL-C level.

Fisher first proposed in 1983 that LDL-C has heterogeneity, which was composed of particles with different sizes and densities.[3] LDL-C was divided into large and light LDL-C (large buoyant low-density lipoprotein cholesterol, lbLDL-C) and small and dense LDL-C (small buoyant) by means of non-denaturing gradient gel scanning, and it was confirmed that the latter was more closely related to the death of acute myocardial infarction.[4] The detection of sdLDL-C (small dense low-density lipoprotein cholesterol) has received widespread attention in clinical and laboratory at home and abroad. The correlation between sdLDL-C and cardiovascular events risk was confirmed by ultracentrifugation, gradient gel electrophoresis, high-performance liquid chromatography, nuclear magnetic resonance, and heparin magnesium precipitation. However, these methods are expensive and time-consuming, which limit the clinical detection of a large number of specimens. Peroxidase detection method is easy to be applied in clinical laboratory, which provides the possibility for clinical routine detection of sdLDL-C.

The sdLDL-C reagent Denka Seiken was the first reagent approved by the Food and Drug Administration (FDA), and its performance has been verified. BSBE was the first sdLDL-C reagents registered by the China Food and Drug Administration, and several studies have shown an association between CVD and sdLDL-C using this reagent. Furthermore, our team has also validated the performance of five other reagents which is Mindray, Medical System, Merit Choice, Dongou and Zybio for sdLDL-C testing. In this study, we evaluate the above seven reagents on two automatic analyzer that are common in Chinese laboratories. To our knowledge, this study is the first to compare these assays of peroxidase method for sdLDL-C measurement.

2 | PATIENTS AND METHODS

2.1 | Patients sample distribution

A total of 336 blood samples were used from patients in June 2019 at Beijing Anzhen Hospital Affiliated to Capital Medical University. These samples comprised 193 males and 143 females. The average age was 31 ~ 87 years old (57.33 ± 10.56 years). The high-value samples were the samples of patients with coronary atherosclerosis, and 326 of them were selected from the comparison samples.

The reference interval was also validated in 298 apparently healthy individuals undergoing medical examination at the same period. According to the prevention and treatment guide of Chinese adult dyslipidemia (2016 Edition), the inclusion criteria were: blood glucose(Glu)<7.0mmol/L, total cholesterol (TC)<5.2 mmol/L, triglyceride (TG)<1.7 mmol/L, high-density lipoprotein cholesterol (HDL-C)≥1.0 mmol/L, low-density lipoprotein cholesterol (LDL-C)<3.4mmol/L. Exclusion criteria: drug abuse, drugs affecting lipoprotein metabolism, drugs for diabetes, hormone replacement therapy, CAD/CHD, diabetes, liver disease, kidney disease, cancer, hospitalization within 6 months.

The blood samples were collected in serum separator tubes (VACUETTE®, Greiner Bio-One GmbH, Austria) and centrifuged at 2860 rcf/g for 10 minutes. All of the samples were stored at −80°C to ensure stability until the analysis time. This study was reviewed and approved by the local Ethics Committee at the Beijing Anzhen Hospital. All of the experimental samples were collected from residual serum samples after routine clinical testing without informed consent.

2.2 | Instrument and reagents

Seven commercially available sdLDL-C assays were analyzed on the Beckman AU5400 (serial number:1 679 334) and Mindray BS2000 (serial number:10.00.04.15569) automatic analyzer. Denka Seiken sdLDL-C reagent (Japan Bio Science Laboratory Co., Ltd., LOT: 468 091), BSBE sdLDL-C reagent (BSBE Co., Ltd., Beijing, China, LOT: 18-0710), Mindray sdLDL-C reagent (Mindray Co., Ltd, LOT: 148 519 001), Medical System sdLDL-C reagent (Medical System Co., Ltd, LOT: 190 121 601), Merit Choice sdLDL-C reagent (Merit Choice Bioengineerign Co., Ltd., Beijing, China, LOT:190 321), Dongou sdLDL-C reagent (Dongou Co., Ltd, LOT: 2019040019m0509), Zybio sdLDL-C reagent (Zybio Co., Ltd, LOT: 190 501) were evaluated. For each assay, the supplied reagents were used, and the calibration methods and experimental assays were performed based on the manufacturer's instructions. All samples were measured only after quality control was measured and confirmed to fall in an acceptable range.

2.3 | Precision

In accordance with the NCCLS EP5-A2 guideline and CLSI EP15-A, precision verification of the seven sdLDL-C assays investigated in this study was performed. The repeatability (coefficients of variation, CV%) was evaluated using two level residual serum samples, and each determination was repeated for 20 times. Total assay precision was evaluated using two different level residual serum samples, and a total of four replicates of each sample were measured for five consecutive days.

TABLE 1 The Within-run CV% of the seven different sdLDL-C assays

Machine	Reagents	Detection times	$\bar{x}$±SD, mmol/L		CV Within-run,%	
			Low-concentration samples	High-concentration samples	Low-concentration samples	High-concentration samples
Mindray BS2000	DENKA SEIKEN	20	0.82 ± 0.02	1.50 ± 0.01	2.23	0.86
	BSBE	20	0.84 ± 0.01	1.29 ± 0.01	0.98	0.81
	Mindray	20	0.89 ± 0.03	1.84 ± 0.02	2.80	1.25
	Zybio	20	0.82 ± 0.02	1.50 ± 0.01	2.23	0.86
	Dongou	20	0.82 ± 0.03	1.85 ± 0.02	3.66	1.19
	Merit Choice	20	0.96 ± 0.02	1.75 ± 0.02	1.85	0.88
	Medical System	20	0.85 ± 0.03	1.77 ± 0.02	2.92	1.07
Beckman AU5400	DENKA SEIKEN	20	0.94 ± 0.03	1.91 ± 0.02	2.75	0.93
	BSBE	20	0.93 ± 0.03	1.87 ± 0.02	3.31	1.14
	Mindray	20	0.93 ± 0.02	1.90 ± 0.02	2.29	1.04
	Zybio	20	1.02 ± 0.02	1.84 ± 0.02	1.62	0.81
	Dongou	20	0.94 ± 0.04	1.88 ± 0.02	3.91	1.01
	Merit Choice	20	1.31 ± 0.02	2.35 ± 0.02	1.80	0.76
	Medical System	20	0.90 ± 0.01	1.40 ± 0.02	1.07	1.13

Note: sdLDL-C concentration is present as the means ± standard deviations. CV Within-run (%) is shown as the percentage of the coefficient of variation.

Abbreviation: CV, coefficient variation.

2.4 | Dilutional linearity

According to the requirements of CLSI EP6-A, dilutional linearity verification was performed by one sample of high value (H) which was close to the upper limit concentration,and the other sample of low value (L) which was the blank sample. Seven concentration of samples were prepared according to the following proportions (6L, 1L + 5h, 2L + 4h, 3L + 3h, 4L + 2h, 5L + 1H, 6h). The measured results (y) and the theoretical value (x) (deviation should be less than 10%) were plotted s for visual inspection and analyzed using linear regression analysis. The lower and upper linearity limits of DENKA SEIKEN, BSBE, Mindray, Zybio, Dongou, Merit Choice and

TABLE 2 The Total CVs of the seven different sdLDL-C assays

Machine	Reagents	$\bar{x}$±SD, mmol/L		CV total,%	
		Low-concentration samples	High-concentration samples	Low-concentration samples	High-concentration samples
Mindray BS2000	DENKA SEIKEN	0.44 ± 0.01	1.18 ± 0.02	1.79	1.72
	BSBE	0.45 ± 0.01	1.17 ± 0.02	2.09	1.96
	Mindray	0.45 ± 0.01	1.18 ± 0.03	2.01	2.89
	Zybio	0.34 ± 0.01	1.15 ± 0.03	2.49	2.42
	Dongou	0.36 ± 0.01	1.18 ± 0.03	2.76	2.37
	Merit Choice	0.40 ± 0.01	1.34 ± 0.03	2.61	2.38
	Medical System	0.59 ± 0.03	1.10 ± 0.02	4.81	1.35
Beckman AU5400	DENKA SEIKEN	0.50 ± 0.02	1.30 ± 0.03	4.22	2.25
	BSBE	0.49 ± 0.02	1.27 ± 0.05	4.31	3.85
	Mindray	0.49 ± 0.02	1.27 ± 0.04	3.90	3.33
	Zybio	0.49 ± 0.05	1.49 ± 0.04	10.33	2.54
	Dongou	0.50 ± 0.02	1.52 ± 0.05	4.23	3.52
	Merit Choice	0.61 ± 0.02	1.89 ± 0.06	3.43	2.93
	Medical System	0.70 ± 0.02	1.25 ± 0.04	3.16	3.44

Note: sdLDL-C concentration is present as the means ± standard deviations. The total CV is shown as the percentage of the coefficient of variation.

Abbreviation: CV, coefficient variation.

TABLE 3 Linearity of the seven different sdLDL-C assays

Machine	Reagents	n	a	b	R^2	r (95%CI)	Average Bias,%	Linearity ,mmol/l
Mindray BS2000	DENKA SEIKEN	7	0.96	0.18	0.982	0.991 (0.984,0.999)	−19.88	0.067 ~ 2.460
	BSBE	7	0.91	0.22	0.969	0.984 (0.969,0.998)	−18.72	0.071 ~ 2.533
	Mindray	7	0.96	0.19	0.981	0.990 (0.984,0.999)	−23.25	0.057 ~ 2.404
	Zybio	7	1.01	0.02	0.998	0.999 (0.999,1.000)	−4.81	0.082 ~ 1.625
	Dongou	7	0.95	0.15	0.981	0.990 (0.982,0.999)	−16.84	0.070 ~ 2.223
	Merit Choice	7	1.00	0.04	0.997	0.999[a] (0.997,1.000)	−5.23	0.185 ~ 2.510
	Medical System	7	0.98	0.05	0.999	0.999 (0.999,1.000)	−3.75	0.210 ~ 2.470
Beckman AU5400	DENKA SEIKEN	7	0.97	0.23	0.975	0.991 (0.984,0.999)	−24.11	0.065 ~ 2.742
	BSBE	7	0.94	0.24	0.976	0.984 (0.968,0.998)	−24.36	0.067 ~ 2.617
	Mindray	7	0.97	0.23	0.977	0.990 (0.984,1.000)	−25.48	0.062 ~ 2.674
	Zybio	7	1.00	3E-05	1.000	0.999 (0.999,1.000)	−0.55	0.097 ~ 1.914
	Dongou	7	0.98	0.17	0.983	0.990 (0.982,0.999)	−21.23	0.076 ~ 2.482
	Merit Choice	7	0.97	0.23	0.977	0.999 (0.997,1.000)	−25.14	0.062 ~ 2.674
	Medical System	7	0.98	0.04	0.999	0.999 (0.999,1.000)	−1.97	0.213 ~ 2.714

Note: 95%CI, confidence intervals of 95%. R^2, coefficient of correlation. The regression line equation is presented as y = ax+b. A, regression line slope. B, regression line intercept.

Medical System were (0.10 ~ 2.59 mmol/L), (0.104 ~ 2.59 mmol/L),(0.104 ~ 2.59 mmol/L),(0.10 ~ 2.33 mmol/L),(0.15 ~ 2.69 mmol/L), (0.21 ~ 2.59 mmol/L), (0.10 ~ 2.59mmol/L), respectively.

2.5 | Recovery

Add sample H to sample L, and the volume of added H is equal to 10% of the total volume (H + L). L is a low-value serum, and H is a high-concentration standard sample close to the upper limit of 50%~70% of the analytical measurement range. Recovery $R=(V_H \times C_{H+L} + V_L \times C_{H+L} - V_L \times C_L)/(V_H \times C_H) \times 100\%$. The measured results shall be within the allowable range, and the deviation shall not exceed 10%.

2.6 | Lower limit of detection

In accordance with the CLSI EP17-A, blank samples were measured continuously for 20 times by the seven sdLDL-C assays on Beckman AU5400 and Mindray BS2000 instruments. The average value of blank samples, added with 3 times standard deviation, was the detection lower limit.

2.7 | Interference test

According to the EP07-P, the interferent were added into low-value mixed serum and high-value mixed serum respectively to make interference serum containing specific quantitative interferent. The final concentration of interfering serum vitamin C was 25, 50(mg/dL), chyle was 300, 600(mg/dL), hemoglobin was 250, 500(mg/dL), and bilirubin was 25, 50 (mg/dL). Seven sdLDL-C reagents were used to measure the interferent serum containing different amount of interferent for three times respectively. The mean value was calculated. The percentage deviation (bias%) between the added interferent and the non-added interferent was calculated. Bias% = (measured value after adding interferent-measured value without adding interferent)/measured value without adding interferent × 100%, and the bias% shall not exceed 10%.

TABLE 4 Sample interferences for common interferents of the seven different sdLDL-C assays

Machine	Interferent, mg/dL	Relative bias, %													
		DENKA SEIKEN		BSBE		Mindray		Zybio		Dongou		Merit Choice		Medical System	
		Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High
Mindray BS2000	Vitamin C														
	25	−0.55	1.45	1.56	1.83	1.75	0.92	−0.20	0.89	2.53	0.44	0.31	−0.85	1.34	0.75
	50	1.70	2.31	3.24	2.33	1.64	1.97	−1.10	2.04	5.34	1.00	1.05	0.46	0.97	1.15
	Chyle														
	300	−2.15	−1.63	−2.19	−2.07	−1.51	−2.69	−7.57	−10.64[a]	−10.72[a]	−9.46	−7.81	−11.19[a]	1.03	−0.08
	600	−5.42	−6.15	−6.70	−5.19	−5.76	−4.38	−27.25[a]	−32.94[a]	−28.85[a]	−26.58[a]	−28.29[a]	−33.81[a]	1.10	−1.28
	Hemoglobin														
	250	−3.99	0.08	−2.46	−0.44	−1.38	−2.02	2.16	1.28	−3.57	−1.97	1.02	1.16	−2.56	1.72
	500	−7.05	−2.66	−3.10	−3.76	−3.16	−3.86	2.57	0.93	−4.52	−4.47	3.19	0.24	0.59	2.20
	Bilirubin														
	25	0.31	−0.36	−0.03	−0.96	0.59	1.13	−20.66[a]	−16.60[a]	−11.82[a]	−12.55[a]	−16.55[a]	−12.67[a]	−12.82[a]	−11.40[a]
	50	−0.08	−0.48	0.42	−2.08	1.60	1.51	−22.37[a]	−21.45[a]	−22.40[a]	−22.91[a]	−17.51[a]	−15.49[a]	−21.95[a]	−18.74[a]
Beckman AU5400	Vitamin C														
	25	1.27	−0.32	−1.35	1.05	0.23	−1.07	−0.44	−1.00	−3.13	−1.70	−0.14	−0.63	−2.15	0.55
	50	3.49	0.30	−2.33	0.66	1.84	0.99	−2.69	0.15	−0.75	−2.09	−0.71	−0.35	−1.64	0.24
	Chyle														
	300	−2.26	−1.34	0.09	−1.41	−1.46	−2.14	−5.17	−4.36	−12.84[a]	−6.27	−3.95	−4.77	0.46	−0.05
	600	−2.74	−4.80	−1.87	−2.89	−4.50	5.83	−20.80[a]	−29.43[a]	−24.95[a]	−25.60[a]	−19.67[a]	−28.06[a]	−0.13	−0.28
	Hemoglobin														
	250	−0.65	−0.68	−5.62	−1.77	−0.30	1.04	1.28	1.75	3.92	1.66	2.55	1.00	2.06	1.75
	500	−4.16	−2.64	−8.99	−4.21	−6.67	−2.79	1.53	2.26	3.92	2.57	2.25	1.50	3.22	1.93
	Bilirubin														
	25	1.17	−0.49	−1.03	0.80	0.13	−0.49	−26.20[a]	−21.82[a]	−11.51[a]	−13.69[a]	−19.30[a]	−17.36[a]	−13.48[a]	−12.97[a]
	50	5.27	1.68	2.58	1.51	2.22	0.78	−26.74[a]	−23.96[a]	−22.19[a]	−22.51[a]	−21.60[a]	−18.55[a]	−23.71[a]	−20.70[a]

[a]the bias% exceeds 10%.Low, low-value mixed serum. High, high-value mixed serum.

TABLE 5 Comparison and concurrence analysis of the six different sdLDL-C assays with DENKA SEIKEN

Machine	Comparison reagent (vs. DENKA SEIKEN)	Pearson's correlation coefficient			Passing and Bablok regression	
		R^2 (*p*-value)	Intercept (95%CI)	Slope (95%CI)	Intercept (95%CI)	Slope (95%CI)
Mindray BS2000	BSBE	0.999 (*p* < .0001)	0.02 (0.005 ~ 0.03)	0.99 (0.98 ~ 1.00)	0.01 (0.005 ~ 0.03)	0.99 (0.98 ~ 1.00)
	Mindray	0.999 (*p* < .0001)	−0.003 (−0.02 ~ 0.10)	0.97 (0.96 ~ 0.98)	−0.004 (0.01 ~ 0.003)	0.97 (0.96 ~ 0.98)
	Zybio	0.968 (*p* < .0001)	0.17 (0.11 ~ 0.22)	0.73 (0.69 ~ 0.76)	0.16 (0.09 ~ 0.21)	0.74 (0.70 ~ 0.78)
	Dongou	0.971 (*p* < .0001)	0.04 (−0.03 ~ 0.10)	0.91 (0.86 ~ 0.95)	0.02 (−0.02 ~ 0.07)	0.92 (0.88 ~ 0.98)
	Merit Choice	0.966 (*p* < .0001)	0.20 (0.14 ~ 0.27)	0.84 (0.80 ~ 0.89)	0.19 (0.10 ~ 0.25)	0.86 (0.81 ~ 0.91)
	Medical System	0.836 (*p* < .0001)	0.34 (0.25 ~ 0.43)	0.51 (0.45 ~ 0.58)	0.26 (0.17 ~ 0.34)	0.56 (0.50 ~ 0.64)
Beckman AU5400	BSBE	0.998 (*p* < .0001)	−0.008 (−0.03 ~ 0.01)	0.99 (0.97 ~ 1.00)	−0.006 (−0.01 ~ 0.04)	0.98 (0.97 ~ 0.99)
	Mindray	0.999 (*p* < .0001)	−0.004 (−0.02 ~ 0.01)	0.99 (0.98 ~ 1.00)	−0.004 (−0.01 ~ 0.004)	0.99 (0.98 ~ 1.00)
	Zybio	0.923 (*p* < .0001)	0.16 (0.05 ~ 0.27)	0.91 (0.83 ~ 0.98)	0.18 (0.08 ~ 0.25)	0.89 (0.83 ~ 0.94)
	Dongou	0.978 (*p* < .0001)	0.04 (−0.02 ~ 0.11)	0.96 (0.92 ~ 1.00)	0.04 (−0.03 ~ 0.09)	0.97 (0.93 ~ 1.02)
	Merit Choice	0.968 (*p* < .0001)	0.27 (0.19 ~ 0.36)	1.09 (1.03 ~ 1.15)	0.24 (0.13 ~ 0.33)	1.12 (1.06 ~ 1.19)
	Medical System	0.826 (*p* < .0001)	0.37 (0.27 ~ 0.48)	0.53 (0.46 ~ 0.60)	0.29 (0.19 ~ 0.36)	0.59 (0.52 ~ 0.68)

2.8 | Reference interval for regent Mindray

The reference interval was validated in 298 apparently healthy individuals according to CLSI C28-A2. If all the test results are within the reference range stated by the manufacturer, or only 5% of the results are beyond, the biological reference range was applicable.

2.9 | Comparison and concurrence analysis

According to the CLSI EP9-A2, DENKA SEIKEN which was the first registered automated homogenous assay in FDA and showed excellent agreement with classic sequential ultracentrifugation,[5] was used as the comparison reagent on two instruments, Mindray BS2000 and Beckman AU5400. Pearson's correlation coefcient was used as a rough estimate of the correlation among the assays. A Pearson's correlation coefficient < 0.95 indicated a low degree of association between two assays. Concurrence among assays was evaluated using Passing and Bablok regression and Bland–Altman plot analysis.

2.10 | Statistics

All statistical analyses were performed using SPSS 22.0 (IBM Inc, Armonk, NY, USA), Microsoft Excel 2017 (Microsoft Corporation,

FIGURE 1 Comparison of sdLDL-C values obtained with the six different sdLDL-C assays and DENKA SEIKEN using Passing and Bablok (PB) regression analysis and Bland–Altman (BA) plot on Mindray BS2000. In the Passing–Bablok regression analyses the orange dotted lines show the 95% confidence interval (CI); the orange solid lines represent the identity line (X = Y); the blue solid lines represent the Passing–Bablok regression line. In the Bland–Altman plots the blue solid lines show the mean difference, while the red dotted lines show the mean difference ± 1.96 SD. A, Comparison of BSBE and Denka Seiken by PB on Mindray BS2000. B, Comparison of BSBE and Denka Seiken by BA on Mindray BS2000. C, Comparison of Mindray and Denka Seiken by PB on Mindray BS2000. D, Comparison of Mindray and Denka Seiken by BA on Mindray BS2000. E, Comparison of Zybio and Denka Seiken by PB on Mindray BS2000. F, Comparison of Zybio and Denka Seiken by BA on Mindray BS2000. G, Comparison of Dongou and Denka Seiken by PB on Mindray BS2000. H, Comparison of Dongou and Denka Seiken by BA on Mindray BS2000. I, Comparison of Merit Choice and Denka Seiken by PB on Mindray BS2000. J, Comparison of Merit Choice and Denka Seiken by BA on Mindray BS2000. K, Comparison of Medical System and Denka Seiken by PB on Mindray BS2000. L, Comparison of Medical System and Denka Seiken by BA on Mindray BS2000

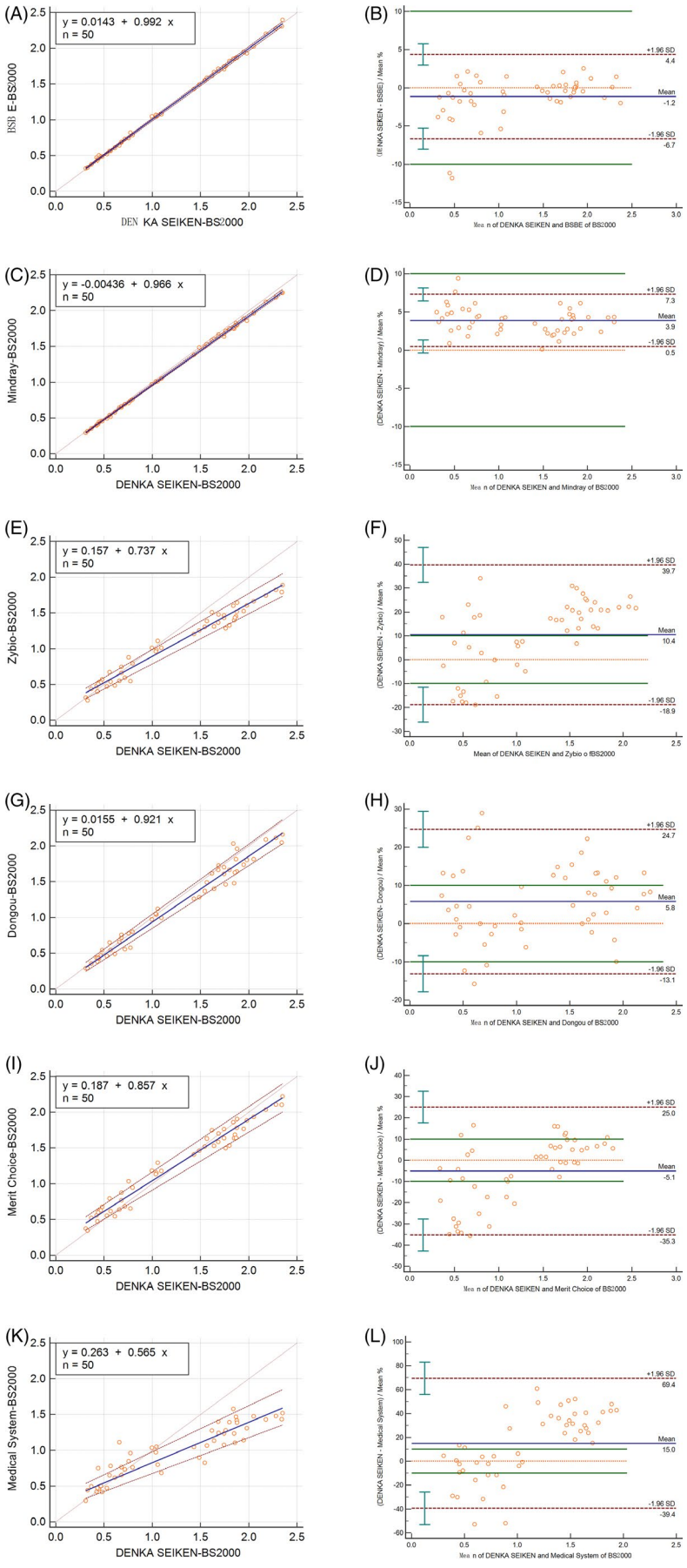

(A)
y = 0.0143 + 0.992 x
n = 50
BSB E-BS2000
DEN KA SEIKEN-BS2000
(B)
+1.96 SD
4.4
Mean
-1.2
-1.96 SD
-6.7
Mean of DENKA SEIKEN and BSBE of BS2000
(C)
y = -0.00436 + 0.966 x
n = 50
Mindray-BS2000
DENKA SEIKEN-BS2000
(D)
+1.96 SD
7.3
Mean
3.9
-1.96 SD
0.5
Mean of DENKA SEIKEN and Mindray of BS2000
(E)
y = 0.157 + 0.737 x
n = 50
Zybio-BS2000
DENKA SEIKEN-BS2000
(F)
+1.96 SD
39.7
Mean
10.4
-1.96 SD
-18.9
Mean of DENKA SEIKEN and Zybio of BS2000
(G)
y = 0.0155 + 0.921 x
n = 50
Dongou-BS2000
DENKA SEIKEN-BS2000
(H)
+1.96 SD
24.7
Mean
5.8
-1.96 SD
-13.1
Mean of DENKA SEIKEN and Dongou of BS2000
(I)
y = 0.187 + 0.857 x
n = 50
Merit Choice-BS2000
DENKA SEIKEN-BS2000
(J)
+1.96 SD
25.0
Mean
-5.1
-1.96 SD
-35.3
Mean of DENKA SEIKEN and Merit Choice of BS2000
(K)
y = 0.263 + 0.565 x
n = 50
Medical System-BS2000
DENKA SEIKEN-BS2000
(L)
+1.96 SD
69.4
Mean
15.0
-1.96 SD
-39.4
Mean of DENKA SEIKEN and Medical System of BS2000

TABLE 6 Bland–Altman analysis of the six different sdLDL-C assays with DENKA SEIKEN

Machine	Bland–Altman (vs. DENKA SEIKEN)	BSBE	Mindray	Zybio	Dongou	Merit Choice	Medical System
Mindray BS2000	Average difference(%) (95%CI)	−1.16 (−1.96~−0.36)	3.87 (3.38 ~ 4.37)	10.40 (6.15 ~ 14.65)	5.78 (3.04 ~ 8.52)	−5.14 (−9.52~−0.76)	15.00 (7.10 ~ 22.89)
	Lower limit (95%CI)	−6.68 (−8.06~−5.30)	0.46 (−0.39 ~ 1.31)	−18.92 (−26.23~−11.60)	−13.12 (−17.84~−8.41)	−35.32 (−42.85~−27.79)	−39.45 (−53.03~−25.87)
	Upper limit (95%CI)	4.35 (2.98 ~ 5.73)	7.29 (6.44 ~ 8.14)	39.71 (32.40 ~ 47.02)	24.68 (19.97 ~ 29.40)	25.04 (17.51 ~ 32.57)	69.45 (55.87 ~ 83.03)
Beckman AU5400	Average difference(%) (95%CI)	2.16 (1.58 ~ 2.74)	1.26 (0.77 ~ 1.75)	−7.14 (−11.61~−2.68)	0.06 (−2.83 ~ 2.95)	−30.85 (−35.11~−26.59)	11.23 (3.10 ~ 19.36)
	Lower limit (95%CI)	−1.84 (−2.84~−0.84)	−2.11 (−2.95~−1.27)	−37.95 (−45.63~−30.26)	−19.89 (−24.87~−14.91)	−60.24 (−67.58~−52.91)	−44.85 (−58.84~−30.86)
	Upper limit (95%CI)	6.16 (5.16 ~ 7.15)	4.63 (3.79 ~ 5.47)	23.66 (15.98 ~ 31.35)	20.01 (15.04 ~ 24.99)	−1.46 (−8.79 ~ 5.87)	67.32 (53.32 ~ 81.30)

USA), and/or MedCalc statistical software (Broekstraat, Mariakerke, Belgium). Continuous data are presented as the means ± standard deviation. Person correlation analysis was used to analyze correlation. The Pearson *r* was used as a rough estimate of the correlation among assays. If the R^2 < 0.95 in the linear regression equation, the methods were not comparable. Concurrence among assays was evaluated using Passing and Bablok regression and Bland–Altman plot analysis. A P < .05 was considered statistically significant.

3 | RESULTS

3.1 | Precision

The repeatability CV% of the seven sdLDL-C assays investigated in this study were 0.81%~3.66% for Mindray BS2000 and 0.76%~3.91% for Beckman AU5400 (Table 1), which were less than the manufacturers' claim (10%), and less than the LDL-C repeatability standard (<7.5%) of EQA as well as the LDL-C repeatability standard (<4%) of blood lipid guide [2016 blood lipid guide], which could meet the clinical application.

Total CVs for Mindray BS2000 sdLDL-C assay were 1.34%~4.80% (Table 2), and that of Beckman AU5400 were 2.25%~10.33% (Table 2), which were less than the manufacturers' claim (13%). Except for Zybio, they were less than the allowable Total CVs' standard of LDL-C in EQA (<10%) and the standard of LDL-C in blood lipid guideline [2016 blood lipid guide] (<5.33%), which could meet the clinical application.

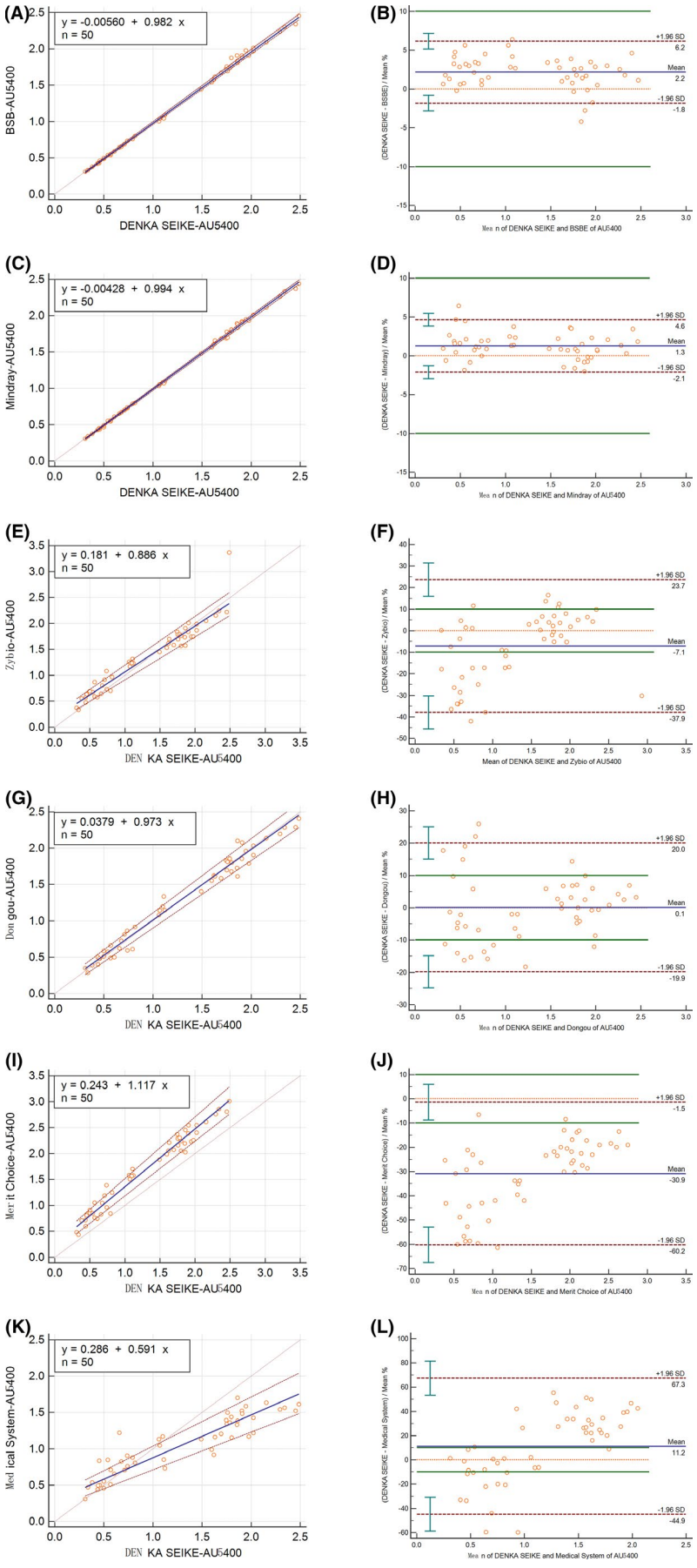
3.2 | Dilutional linearity

The determination coefficients of regression equation, R^2 and lower and upper linearity for the assay are shown in table 3. Visual inspection and regression analysis demonstrate that only Zybio and Medical System are linear over the range examined both on Mindray BS2000 and Beckman AU5400. Merit Choice is linear over the range examined on Mindray BS2000.

3.3 | Recovery test

The average recovery rates of sdLDL-C assays for DENKA SEIKEN, BSBE, Mindray, Zybio, Dongou, Merit Choice and Medical System on Beckman AU5400 were 92.56%, 92.33%, 91.95%, 98.23%, 92.65%, 98.76% and 98.33% respectively. The average recovery rates of sdLDL-C assays for DENKA SEIKEN, BSBE, Mindray, Zybio, Dongou, Merit Choice and Medical System on Mindray BS2000 were 93.68%, 92.29%, 93.51%, 93.51%,

FIGURE 2 Comparison of sdLDL-C values obtained with the six different sdLDL-C assays and DENKA SEIKEN using Passing and Bablok (PB) regression analysis and Bland–Altman (BA) plot on Beckman AU5400. In the Passing–Bablok regression analyses the orange dotted lines show the 95% confidence interval (CI); the orange solid lines represent the identity line (X = Y); the blue solid lines represent the Passing–Bablok regression line. In the Bland–Altman plots the blue solid lines show the mean difference, while the red dotted lines show the mean difference ± 1.96 SD. A, Comparison of BSBE and Denka Seiken by PB on Beckman AU5400. B, Comparison of BSBE and Denka Seiken by BA on Beckman AU5400. C, Comparison of Mindray and Denka Seiken by PB on Beckman AU5400. D, Comparison of Mindray and Denka Seiken by BA on Beckman AU5400. E, Comparison of Zybio and Denka Seiken by PB on Beckman AU5400. F, Comparison of Zybio and Denka Seiken by BA on Beckman AU5400. G, Comparison of Dongou and Denka Seiken by PB on Beckman AU5400. H, Comparison of Dongou and Denka Seiken by BA on Beckman AU5400. I, Comparison of Merit Choice and Denka Seiken by PB on Beckman AU5400. J, Comparison of Merit Choice and Denka Seiken by BA on Beckman AU5400. K, Comparison of Medical System and Denka Seiken by PB on Beckman AU5400. L, Comparison of Medical System and Denka Seiken by BA on Beckman AU5400


(A)
y = -0.00560 + 0.982 x
n = 50
BSB-AU5400
DENKA SEIKE-AU5400
(B)
+1.96 SD
6.2
Mean
2.2
-1.96 SD
-1.8
(DENKA SEIKE - BSBE) / Mean %
Mean of DENKA SEIKE and BSBE of AU5400
(C)
y = -0.00428 + 0.994 x
n = 50
Mindray-AU5400
DENKA SEIKE-AU5400
(D)
+1.96 SD
4.6
Mean
1.3
-1.96 SD
-2.1
(DENKA SEIKE - Mindray) / Mean %
Mean of DENKA SEIKE and Mindray of AU5400
(E)
y = 0.181 + 0.886 x
n = 50
Zybio-AU5400
DENKA SEIKE-AU5400
(F)
+1.96 SD
23.7
Mean
-7.1
-1.96 SD
-37.9
(DENKA SEIKE - Zybio) / Mean %
Mean of DENKA SEIKE and Zybio of AU5400
(G)
y = 0.0379 + 0.973 x
n = 50
Dongou-AU5400
DENKA SEIKE-AU5400
(H)
+1.96 SD
20.0
Mean
0.1
-1.96 SD
-19.9
(DENKA SEIKE - Dongou) / Mean %
Mean of DENKA SEIKE and Dongou of AU5400
(I)
y = 0.243 + 1.117 x
n = 50
Merit Choice-AU5400
DENKA SEIKE-AU5400
(J)
+1.96 SD
-1.5
Mean
-30.9
-1.96 SD
-60.2
(DENKA SEIKE - Merit Choice) / Mean %
Mean of DENKA SEIKE and Merit Choice of AU5400
(K)
y = 0.286 + 0.591 x
n = 50
Medical System-AU5400
DENKA SEIKE-AU5400
(L)
+1.96 SD
67.3
Mean
11.2
-1.96 SD
-44.9
(DENKA SEIKE - Medical System) / Mean %
Mean of DENKA SEIKE and Medical System of AU5400

96.46%, 99.12% and 99.44%, respectively. The measured recovery rates of sdLDL-C assays were within the allowable ± 10% deviation range.

3.4 | Lower limit of detection

The LLD of sdLDL-C assays for DENKA SEIKEN, BSBE, Mindray, Zybio, Dongou, Merit Choice and Medical System on Beckman AU5400 was 0.0021, 0.0016, 0.0019, 0.0019, 0.0023, 0.0041, 0.0020, respectively. The LLD of sdLDL-C assays for DENKA SEIKEN, BSBE, Mindray, Zybio, Dongou, Merit Choice and Medical System on Mindray BS2000 was 0.0022, 0.0019, 0.0016, 0.0011, 0.0012, 0.0022, 0.0005, respectively. There was no obvious difference in the lower limit of each manufacturer's detection, which was less than the manufacturer's lower limit of detection, meeting the basic detection requirements.

3.5 | Interference test

The anti-hemolysis and anti-vitamin C interference ability of all sdLDL-C assays were acceptable and met the requirement stated by the manufacturers. The interference of chyle were acceptable among DENKA SEIKEN, BSBE, Mindray, and Medical System. DENKA SEIKEN, BSBE and Mindray showed good anti-interference ability to bilirubin. Medical System was negatively affected by bilirubin, the maximum deviation of which was −23.71% on Beckman AU5400 and −21.95% on Mindray BS2000. Zybio and Dongou were negatively affected by chyle, the maximum deviation of which were −29.43% and −25.60% on Beckman AU5400, as well as −32.49% and −28.85% on Mindray BS2000. Merit Choice was negatively affected by chyle and bilirubin, the maximum deviation of which were −28.06% and −21.60% respectively on Beckman AU5400,as well as −33.81% and −17.51% respectively on Mindray BS2000. (Table 4).

3.6 | Reference interval for regent Mindray

There were 214 cases (71.81%) whose results fell within the reference range stated by the manufacturer, and 84 cases (28.19%) whose low value was lower than the lower limit of the reference range. The $\bar{x}$±SD of 298 samples was 0.33 ± 0.14 (mmol/L), and the reference range established by using $\bar{x}$±2SD was 0.05 ~ 0.61 (mmol/L). According the limit of ± 15%, there was a difference between the validation results of the reference range and the manufacturer's (0.243 ~ 1.393 mmol/L), indicating that the biological reference range was not applicable to our laboratory.

3.7 | Comparison and concurrence analysis

Pearson's correlation coefficients, intercept and slope of the six different sdLDL-C assays with DENKA SEIKEN were showed in Table 5. BSBE, Mindray, and Dongou had high degree of association with DENKA SEIKEN on Mindray BS2000, while BSBE, Mindray, Dongou and Merit Choice had high degree of association with DENKA SEIKEN on Beckman AU5400.

On Passing and Bablok regression analysis, the 95% confidence interval (CI) for the intercept did not contain 0, while the 95% CI for slope contained 1, only in one regression equations which was BSBE on Mindray BS2000 (Table 5, Figure 1).

The Bland–Altman plot was used to compare and evaluate the results of different assays. If more than 95%of the points within the 95% consistency limit, and the biases (%) were less than 10%, BSBE and Mindray on Mindray BS2000 (Table 6, Figure 1) as well as Beckman AU5400(Table 6, Figure 2) were meeting the requirements.

4 | DISCUSSION

Small and dense low-density lipoprotein cholesterol (sdLDL-C) is a common type of low-density lipoprotein cholesterol (LDL-C).[3] Research shows that sdLDL-C is the main subtype of atherosclerosis, and it is closely related to the occurrence of cardiovascular and cerebrovascular events in atherosclerotic diseases, which has a high-reference value for the evaluation of cardiovascular and cerebrovascular diseases.[6-9] At present, there are many detection methods of sdLDL-C, and peroxidase detection is one of them. Compared with gradient gel electrophoresis, density gradient ultracentrifugation and nuclear magnetic resonance spectroscopy, the method of peroxidase detection is easy to be applied in clinical laboratory, which provides the possibility for clinical routine detection of sdLDL-C.[10-12]

The detection principle of peroxidase sdLDL-C kit comes from Hirano's theory that the specific reaction between special surfactants and lipoproteins can be used to detect the level of sdLDL-C.[13] In other words, polyoxyethylene benzyl phenyl ether derivatives were selected as surfactants to selectively dissociate non-LDL-C lipoproteins. Cholesterol esters were decomposed by cholesterol esterase and cholesterol oxidase, while large and light low-density lipoproteins were hydrolyzed by sphingomyelinase, so only the remaining sdLDL-C participated in the color reaction. In this method, the enzyme in the reagent can react with specific lipoproteins with specific surfactants. sdLDL-C is released to participate in the principle of color reaction for detection, which has strong operability.[14]

The repeatability CV% of the seven sdLDL-C assays investigated in this study were 0.81%~3.66% for Mindray BS2000 and 0.76%~3.91% for Beckman AU5400, while Total CVs for Mindray BS2000 sdLDL-C assay were 1.34%~4.80%, and that of Beckman AU5400 were 2.25%~10.33%. The measured recovery rates of sdLDL-C assays were within the allowable ±10% deviation range. There was no obvious difference in the lower limit of each manufacturer's detection, which is consistent with CLSI guidelines, suggesting the value of peroxidase assay. Such discrepancy may be due to the different sdLDL-C reagent composition or traceability.

Biological reference interval is the basic scale and basis for the interpretation of test results and analysis of test information, and it

is also the basic problem in clinical medicine. The laboratory should provide a reliable reference range for the test items, so that the clinic can have a general understanding of the test results of patients and play the role of the test report.[15] The reference interval was validated in 298 apparently healthy individuals according to CLSI C28-A2.There were 214 cases (71.81%) whose results fell within the reference range stated by the manufacturer, and 84 cases (28.19%) whose low value was lower than the lower limit of the reference range, indicating that the biological reference range was not applicable to our laboratory.

Although our research has reached its aims of comparing commonly used sdLDL-C assays, this study does have some limitations. For instance, although sdLDL-C is a newly introduced clinical project in recent years, it is difficult to obtain the reference materials for accuracy verification, so this experiment did not carry out the accuracy verification. It is hoped that the project can be improved Correctness verification test of with the further application.

Our results indicate that the basic performance can meet the testing requirements, but the comparability between them is still insufficient.

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CONFLICT OF INTEREST

The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

AUTHOR CONTRIBUTIONS

All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the corresponding author upon request.

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