Clin. Lab. 2023;69:XXX-XXX

ORIGINAL ARTICLE

Evaluation of PA990pro, an Analyzer for C-Reactive Protein Using Whole Blood: Hematocrit Correction is Required

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SUMMARY

Background: **The rapid detection of C-reactive protein (CRP) in whole blood can provide a basis for reducing the use of antibiotics, especially for infants in whom blood collection is difficult. Whether the performance of PA990pro for CRP detection can meet clinical needs has not been studied.**
Methods: **Between May and June 2022, 230 blood samples were collected to explore the analytical performance of the PA990pro for CRP detection. The blank check, carryover, repeatability, intermediate precision, linearity, sample stability, the influence of hematocrit (HCT)/triglyceride/bilirubin, and the trueness of the PA990pro were evaluated. Whole blood CRP test results analyzed using the PA990pro were compared to plasma CRP test results from a Hitachi 7180 biochemical analyzer, using the same samples.**
Results: **The blank check (≤ 0.3 mg/L), carryovers (≤ 0.05%), repeatability (≤ 7.23%), and intermediate precision (≤ 7.36%) can meet clinical needs. The linear correlation coefficients of different ranges of CRP were good (r >0.975), and the slopes were all within 0.950 - 1.050. The stability of samples was good within 72 hours whether stored at 18 - 25℃ or 2 - 8℃ (CV < 10%). With interference from triglycerides at ≤ 7 mmol/L, the deviation of CRP was < 10% and with bilirubin at ≤ 216 μmol/L, the deviation of CRP was < 10%. PA990pro has no HCT quantification function; abnormal HCT will significantly interfere with whole blood CRP results (the relative deviation in the basic experiment was up to 73.71%). We suggest that the HCT results of the patient during the same period are provided through the laboratory information system (LIS) so that a CRP correction formula ($CRP_{corrected} = CRP_{measured}*(1 - 40\%)/(1 - HCT_{measured})$) can be used on the LIS. After using the formula to perform the HCT correction, the results of PA990pro were well correlated with the 7180 analyzer plasma CRP detection (r > 0.975). PA990pro could pass the external quality assessment of the National Center for Clinical Laboratories.**
Conclusions: **The CRP detection performance of PA990pro can meet clinical needs, but it is suggested that HCT should be corrected by the formula set in LIS. It is a simple, rapid, and cost-free approach that can be used to obtain a modified whole blood CRP test result that meets clinical needs.**
(Clin. Lab. 2023;69:xx-xx. DOI: 10.7754/Clin.Lab.2022.221005)

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Manuscript accepted November 20, 2022

KEYWORDS

C-reactive protein, performance evaluation, hematocrit, correction

INTRODUCTION

C-reactive protein (CRP) is an acute phase reaction protein. During infection, trauma, or tissue injury, the body releases inflammatory factors to induce the synthesis and secretion of CRP from the liver [1]. CRP begins to

rise 4 - 6 hours after the onset of inflammation, doubling every 8 hours, and reaching a peak at 36 - 50 hours [2]. The half-life of CRP is very short (4 - 7 hours); CRP will decrease rapidly once the inflammation resolves. CRP is less affected by drugs and physiological factors, so it can be used as an auxiliary diagnostic indicator of infectious and inflammatory diseases; as such, it is one of the most common infection markers in clinical practice [3].

With the development of detection technologies, whole blood CRP detection can replace serum CRP detection due to its rapidity (5 minutes), smaller sample volume requirements (10 μL), and other advantages. In children with fever, from whom blood is relatively difficult to collect, and febrile patients in the emergency department, whole blood CRP detection provides an important clinical tool for identifying infection and a need for antibiotic treatment. However, research is needed to address whether the instrument performance of a whole blood CRP detection method meets clinical requirements, identify the main interference factors, and answer whether this approach is comparable to CRP detection by a traditional biochemical analyzer. This study systematically evaluates the CRP detection performance of the PA990pro, the latest generation of specific protein analyzers, to provide accurate and reliable detection results for clinical practice.

MATERIALS AND METHODS

Materials

Specimen Collection

Two hundred fresh venous whole blood samples (anticoagulated with EDTA-K_2) and 30 serum samples were collected from the clinical laboratory of Beijing Jishuitan Hospital from May to June 2022. Blood samples were collected according to the Clinical Laboratory Standards Institute (CLSI) EP28 - A3c standard [4].

Instruments and reagents

We used an XN2800 hematology analyzer and CRP assembly line. The assembly line included an XN - A1 automatic hematology analyzer (Sysmex, Japan), XN - B4 automatic hematology analyzer (Sysmex, Japan), and a PA990pro specific protein analyzer (Lifotronic, China). The blood cell analysis reagent and CRP reagent (Immunoturbidimetry) were corollary reagents. We also used a Hitachi 7180 Biochemical analyzer (Hitachi, Japan) and CRP kit (Immunoturbidimetry, Sichuan Maccura, China). The matched calibrators and quality controls were used for corresponding instruments. The PA-990pro was tested with Roche composite quality control (Roche, Germany) in addition to the original CRP quality control (Lifotronic, China).

Interfering substances

Triglycerides were purchased from Fresenius Kabi Corporation (China). Bilirubin was purchased from Sigma Corporation (USA).

The external quality assessment samples

The external quality assessment samples of CRP with the values of 50.452 mg/L, 13.739 mg/L, 72.443 mg/L, 28.409 mg/L, and 82.143 mg/L were provided by the National Center for Clinical Laboratories.

Methods

Blank determination

Sample dilutions were used as specimens for the analyzer. Three consecutive tests were performed and the maximum value of the three test results was recorded.

Carryover contamination

According to the CLSI H26 - A2 carryover contamination rate assessment method, high CRP whole blood samples (CRP > 80 mg/L) and low CRP whole blood samples (CRP < 10mg/L) were selected. Three high-value samples were measured on the instrument being tested, then three low-value samples were measured. Then, we calculated the carryover contamination rate [5].

Reproducibility

Ten whole blood samples of CRP < 10 mg/L, 10 - 30 mg/L, and > 30 mg/L EDTA-K_2 anticoagulant were selected. Each sample was tested 10 consecutive times on the instrument being tested. The mean value, standard deviation (SD), and coefficient of variation (CV) of each concentration range were calculated.

Intermediate precision

According to the CLSI EP05 - A3 precision assessment method, each level of quality control substance was tested twice every morning as the result of the first batch. At an interval of at least 2 hours, the quality control materials of each level were tested twice again as the results of the second batch of tests. Between the two batches, at least 10 patient samples were measured. Measurements were continuously conducted for twenty days. If any of the quality control results exceed the deviation limit, the underlying reason was investigated and corrected, and the precision assessment test was carried out only when the quality control was found to be within the permissible range. The intermediate precision was calculated from test results obtained over 20 days [6].

Linear range

According to the "WS/T420 - 2013 Clinical Laboratory Verification of Analytical performance of commercial quantitative Kit" linear evaluation method, high concentration serum samples close to the upper limit of the linear range and low concentration serum samples close to the lower limit of the linear range were diluted

into 7 different concentration gradient samples (100%, 80%, 60%, 40%, 20%, 10%, and 0). Each dilution was then tested 3 times. CLSI EP06-A was used for piecewise linear statistics, and the three segments were 0.3 - 10.0 mg/L, 10.1 - 100.0 mg/L, and 100.1 - 220.0 mg/L [7].

Sample stability

Ten fresh anticoagulated venous whole blood samples (CRP < 10 mg/L in 5 cases, CRP 10 - 50 mg/L in 3 cases, CRP 50 - 100 mg/L in 1 case, and CRP > 100 mg/L in 1 case) were selected according to the international committee for blood standardization 2014 hematology analyzer evaluation guidelines. The volume of each sample was ≥ 2 mL. Each sample was divided into 13 equal aliquots of approximately 150 μL; each was placed in an EP tube. The first sample tube was measured twice 30 minutes after collection. The remaining 12 samples were divided into 2 groups, 6 aliquots were stored at room temperature (18 - 25℃) and 6 were refrigerated (2 - 8℃) [8]. One aliquot from each temperature group was tested twice at the 4^{th}, 8^{th}, 12^{th}, 24^{th}, 48^{th}, and 72^{nd} hour. The refrigerated samples were returned to room temperature before testing. Test results at 0.5 hours were used as controls, and the changes of each parameter over time were calculated and expressed as relative deviation.

Effect of hematocrit (HCT) on whole blood CRP test results, correction, and verification

Influence of hematocrit (HCT) on the results of a whole blood CRP test

Five samples with CRP > 30.00 mg/L were selected and centrifuged at 800 g for 10 minutes at 18 - 25℃. After centrifugation, the upper plasma layer was aspirated, and the HCT of the lower layer was quantified using a hematology analyzer (the target of the HCT was 70% - 75%). For each centrifugal sample, the upper layer of plasma was defined as diluted plasma for sample, and the lower layer was defined as the HCT high-value sample. The diluted plasma and HCT high-value sample were proportionally mixed to obtain 6 different HCT concentrations, of which the volume proportion of the HCT high-value samples were 10%, 20%, 40%, 60%, 80%, and 100%. The relative deviation between the CRP detection in samples with different concentrations of HCT and the 40% volume proportion of high-value samples were calculated.

CRP correction based on the measured values of HCT

In clinical practice, CRP concentration refers to the serum or plasma concentration. To remain consistent with clinical practice, when whole blood samples are used for detection, the test results need to be converted to obtain the equivalent serum or plasma CRP concentration of the specimen. To solve the influence of HCT fixed at 40% on the whole blood CRP results, we converted the whole blood value by using a modified formula according to the measured HCT value obtained from the blood cell analysis.

The conversion formula is as follows: $CRP_{corrected} = CRP_{measured} * (1 - 40\%)/(1 - HCT_{measured})$, where $CRP_{corrected}$ is the CRP concentration corrected by the measured HCT from a clinical blood cell analysis; $CRP_{measured}$ is the CRP concentration obtained from Lifotronic PA990pro (the default HCT was fixed at 40%); $HCT_{measured}$ is the true HCT value obtained from a clinical blood cell analysis.

Hematocrit is the volume percentage of red blood cells in whole blood. In whole blood samples, a smaller volume of plasma is associated with a higher proportion of red blood cells (higher hematocrit). Forty-four cases of HCT < 35%, 52 cases of HCT 35% - 45%, and 40 cases of HCT > 45% EDTA-K_2 anticoagulated whole blood samples were selected. Some of the blood samples were randomly selected and centrifuged at 800 g for 10 minutes at 18 - 25℃, and the upper part of the plasma was aspirated to improve the HCT value of the sample. The 136 samples were measured by an XN - A1 hematology analyzer to obtain the true HCT value, which was used to correct the Lifotronic PA990pro whole blood CRP test results. Subsequently, the above whole blood samples were centrifuged at 3,500 g for 10 minutes to obtain plasma samples. The plasma samples were used for CRP testing on a Hitachi 7180 biochemical analyzer. We then evaluated the comparability of CRP test results between the Lifotronic PA990pro and Hitachi 7180 Biochemical analyzer under different HCT ranges with or without HCT correction.

The interference of triglycerides (TG) on whole blood CRP

Triglycerides were prepared with seven different concentrations (100%, 80%, 60%, 40%, 20%, 10%, and 5%). The highest concentration of TG was approximately 70 mmol/L. Five samples of EDTA-K_2 anticoagulated blood were randomly selected. Each sample was divided into 8 equal aliquots, each containing 100 μL of whole blood. The first aliquot was mixed with 100 μL 0.9% normal saline; the remaining aliquots were mixed with 100 μL of the various triglyceride concentrations. All samples were tested for CRP 3 times to compare the relative deviation between the TG - interfering CRP results and the 0.9% normal saline - interfering CRP results.

The interference of bilirubin on whole blood CRP

Bilirubin was prepared in five concentrations (100%, 80%, 60%, 40%, and 20%); the highest concentration of bilirubin was approximately 432 μmol/L. Five samples of EDTA-K_2 anticoagulated blood were randomly selected. Each sample was divided into 6 equal aliquots of 100 μL of whole blood. The first aliquot was mixed with 100 μL 0.9% normal saline; the remaining aliquots were mixed with 100 μL of the various bilirubin concentrations. All samples were tested for CRP 3 times to compare the relative deviation between the bilirubin -

interfering CRP results and the 0.9% normal saline-interfering CRP results.

Correctness verification
CRP serum samples at the 5 concentrations (within the reportable range) were assigned as 50.452 mg/L, 13.739 mg/L, 72.443 mg/L, 28.409 mg/L, and 82.143 mg/L, were measured on different instruments for 5 consecutive days, and each was measured twice a day.
The mean value and offset value of each instrument were calculated. If the measurement offset value of the correctness verification material was less than the uncertainty of the value assigned to the correctness verification material, the verification was deemed to have passed, and there was no need to calculate the validation interval. If the measurement offset value of the correctness verification material was greater than the uncertainty of the value assigned to the correctness verification material, then the verification interval of this offset value required to be calculated and compared with the assigned value. If the assigned value was within the verification interval, the verification was deemed to have passed. If the assigned value exceeded the validation interval, the validation was deemed to have failed.

Statistics
SPSS 19.0, Excel 2007, and Medcalc 9.2.1.0 software were used for data analysis and mapping. Passing - Bablok regression and Bland - Altman plot were used to analyze the correlation, consistency, and bias between the two methods. If the 95% CI of the slope of the Passing - Bablok regression did not include 1, we considered this to indicate that there were proportional systematic errors between the two detection methods. If the 95% CI of the intercept did not include 0, it indicated that there were constant systematic errors between the two detection methods. p-values < 0.05 were considered to be statistically significant.

RESULTS

Blank test
The blank test results of the detection system were less than 0.3 mg/L, which met the requirements of the manufacturer's instructions.

Carryover contamination
The carryover contamination rate of the system was as low as 0.05% and enough to meet the requirements of the manufacturer's instructions and the clinical needs.

Repeatability
When the CRP concentration range was 3 - 10 mg/L, the selected 10 samples were repeated 10 times separately, and the CV was found to be < 7.23%. When the CRP concentration range was 10 - 30 mg/L, the CV was < 6.88%. When the CRP concentration range was more than 30 mg/L, the CV was < 6.13%. All results met the industry standard of CVs < 10%.

Intermediate precision
PA990pro uses Lifotronic original quality control materials to detect low-level quality controls (target value 25 mg/L; the intermediate precision was 4.65%) and high-level quality controls (target value 142 mg/L; the intermediate precision was 7.36%). The intermediate precision of the PA990pro was 2.52% for low-level quality controls (target value 10 mg/L) and 3.74% for high-level quality controls (target value 50 mg/L) using Roche multiple composite quality control materials.

Linear range
The slope ranged from 0.950 to 1.050 in the three linear tests (0.3 - 10.0 mg/L, 10.1 - 100.0 mg/L, and 100.1 - 220.0 mg/L). The R^2 values were all > 0.95, as shown in Figure 1A - 1C.

Sample stability
The CRP results of whole blood samples were stable within 72 hours whether they were stored at room temperature (18 - 25℃) or refrigerated (2 - 8℃); the relative deviation was less than 10%. As shown in Figure 2.

Effect of HCT on whole blood CRP test results
Influence of hematocrit (HCT) on the results of the whole blood CRP test
PA990pro has no whole blood HCT detection function, and the results were significantly affected by different concentrations of HCT. The relative deviation of whole blood CRP detection results between the different dilution concentrations of HCT and 40% dilution concentration points of the sample was up to 73.71% (Table 1).

HCT real-time corrected whole blood CRP test results compared with Hitachi 7180 plasma CRP test results
Correction for HCT < 35%
Passing-Bablok regression analysis showed that the slope was 1.3292 (95% CI: 1.2458 to 1.4126, p < 0.0001) between the CRP test results from the 7180 plasma and the CRP test results from the PA990pro when the HCT was fixed at 40%. This suggests that there was a proportional systematic error between the two methods. The intercept was -0.4188 (95% CI: -5.8022 to 4.9647, p = 0.8760), indicating that there was no constant systematic error between the two methods. Details are provided in Figure 3a. The slope between the CRP test results from the 7180 plasma and the real - time HCT modified CRP test results of the PA990pro was 1.1004 (95% CI: 1.0322 to 1.1686, p < 0.0001). The intercept was -0.1025 (95% CI: -4.5060 to 4.3011, p = 0.9628). For details see Figure 3A. In general, when the whole blood HCT was < 35%, the results from the 7180 plasma and the results of the whole blood CRP test with or without HCT correction showed a good correlation (r > 0.975); the real-time HCT corrected whole

Table 1. Relative deviation between the CRP detection of the different sample dilutions and the CRP detection results at 40% dilution concentration points.

Samples	Volume proportion of the HCT high-value samples				
	10%	20%	60%	80%	100%
Sample 1 (166.05 mg/L)	45.02%	30.79%	-25.84%	-42.16%	-61.09%
Sample 2 (85.905 mg/L)	19.34%	13.87%	-21.43%	-43.28%	-65.49%
Sample 3 (57.025 mg/L)	24.91%	22.00%	-30.78%	-44.53%	-71.95%
Sample 4 (48.88 mg/L)	35.70%	22.52%	-25.29%	-49.64%	-69.10%
Sample 5 (35.21 mg/L)	28.60%	23.12%	-26.14%	-53.59%	-73.71%

Table 2. Relative deviation of CRP test results between samples with different concentrations of triglyceride and samples without triglyceride.

Samples	Final Triglyceride concentration (mmol/L)						
	35	28	21	14	7	3.5	1.85
Sample 1 (9.67 mg/L)	20%	19%	17%	16%	1%	4%	-2%
Sample 2 (21.04 mg/L)	20%	12%	13%	11%	10%	8%	9%
Sample 3 (35.22 mg/L)	12%	12%	8%	2%	-1%	1%	6%
Sample 4 (250.92 mg/L)	-9%	-5%	-1%	0%	-1%	-3%	2%
Sample 5 (337.09 mg/L)	-10%	2%	6%	7%	-2%	1%	2%

Table 3. Relative deviation of CRP test results between samples with different concentrations of bilirubin and samples without bilirubin.

Samples	Final Bilirubin concentration (μmol/L)				
	43.2	86.4	129.6	172.8	216
Sample 1 (201.63 mg/L)	-2.06%	1.67%	4.06%	6.39%	1.12%
Sample 2 (30.66 mg/L)	-2.97%	-1.57%	-4.22%	-3.20%	-5.13%
Sample 3 (20.02 mg/L)	-3.76%	-9.74%	-8.67%	-4.07%	-4.36%
Sample 4 (18.24 mg/L)	2.43%	0.88%	0.27%	0.20%	-0.97%
Sample 5 (9.63 mg/L)	2.15%	2.35%	6.06%	8.30%	8.72%

Table 4. Correctness verification of CRP measured by PA990pro.

Proficiency testing samples	Measurement of the mean (mg/L)	Specify a value (mg/L)	bias (%)	Acceptable range (mg/L)	Conclusion
Level 1	47.51	50.452	-5.831	37.839 - 63.065	pass
Level 2	14.40	13.739	4.811	10.304 - 17.174	pass
Level 3	65.66	72.443	-9.363	54.332 - 90.554	pass
Level 4	27.63	28.409	-2.742	21.307 - 35.511	pass
Level 5	75.02	82.143	-8.671	61.607 - 102.679	pass

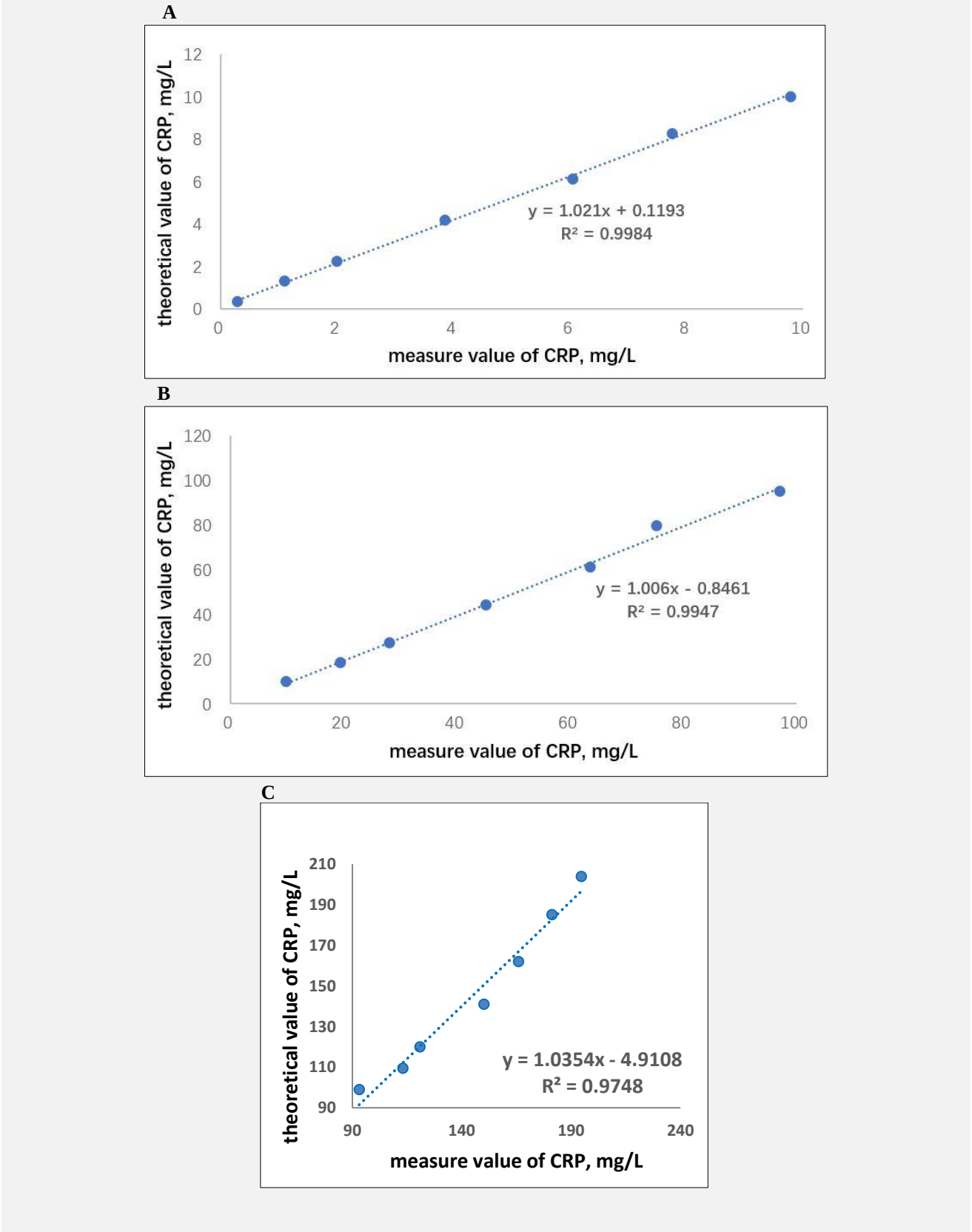

Figure 1. Linear range of CRP low (0.3 - 10.0 mg/L), medium (10.1 - 100.0 mg/L), and high (100.1 - 220.0 mg/L).

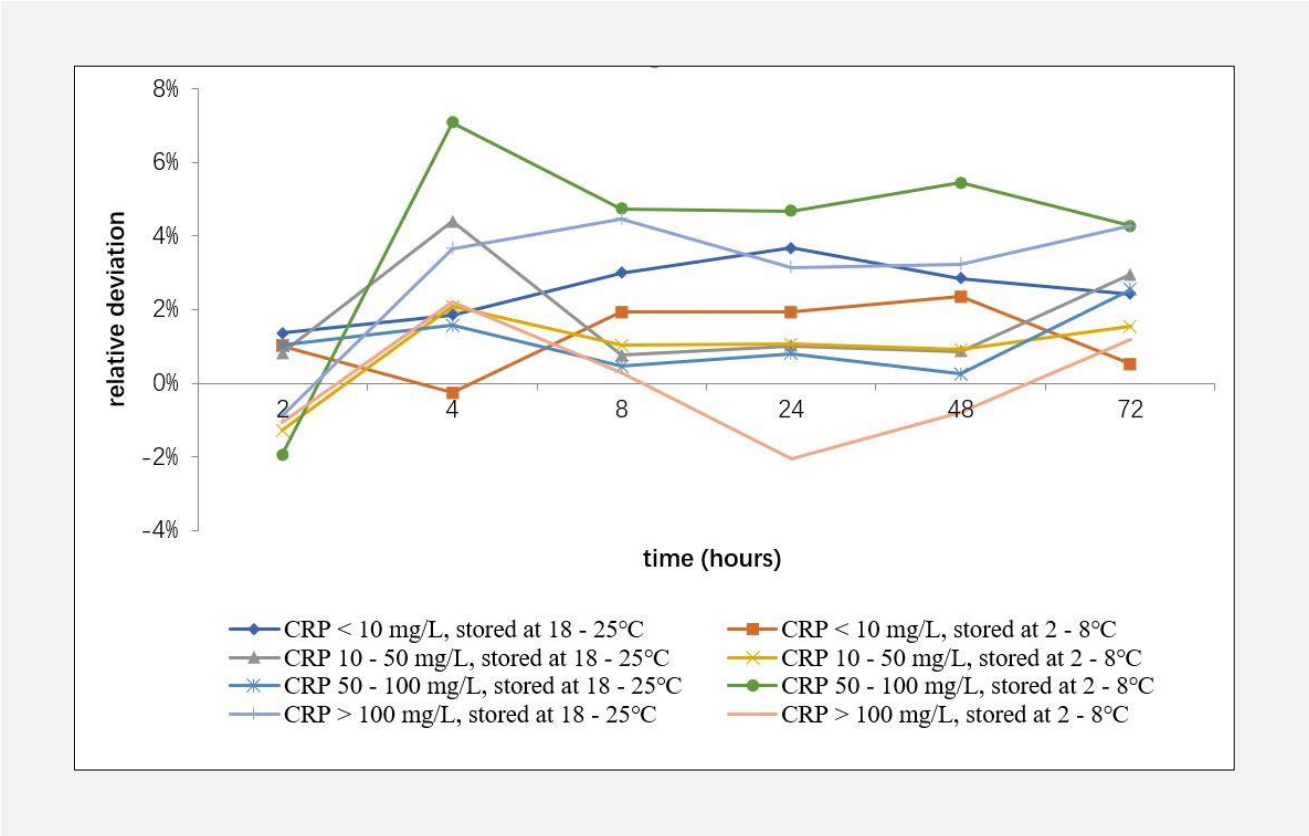

Figure 2. Stability of CRP assay results of different concentrations of whole blood samples at room temperature and refrigerated conditions.

blood CRP test results could significantly reduce the proportional systematic error between the 7180 plasma and the PA990pro whole blood CRP tests.

Bland-Altman plots showed that: compared to HCT fixed at 40%, the average relative deviation between HCT real-time corrected whole blood CRP and the 7180 plasma CRP decreased significantly from -25.4808% (95% CI: -33.4266% to 17.5351%) to -5.2520 % (95% CI: -10.9763% to 0.4273%). For details see Figures 3b and 3B.

Correction for HCT 35% - 45%

The results of Pasing-Bablok regression analysis showed that the slope between the CRP test results from the 7180 plasma and the CRP test results from the PA-990pro when the HCT was fixed at 40% was 1.2407 (95% CI: 1.1986 to 1.2828, $p < 0.0001$). This suggests that there was a proportional systematic error between the two methods. The intercept was -1.6035 (95% CI: -2.6819 to 0.5251, $p = 0.0044$), suggesting that there was a constant systematic error between the two methods. For details see Figure 4a. The slope between the CRP test results from the 7180 plasma and the real-time HCT modified CRP test results from the PA990pro was 1.2453 (95% CI: 1.2097 to 1.2810, $p < 0.0001$). The intercept was -1.7817 (95% CI: -2.6960 to 0.8674, $p = 0.0003$). For details see Figure 4A. In short, when the whole blood HCT was 35% - 45%, the correlation between the 7180 plasma test and the PA990pro whole blood CRP test with or without HCT correction was good ($r > 0.975$).

Bland-Altman plots showed that, compared with HCT fixed at 40%, the average relative deviation between the 7180 plasma CRP and the PA990pro real-time HCT corrected whole blood CRP decreased from -2.1173 % (95% CI: -8.4258% to 4.1912%) to -1.4023 % (95% CI: -7.7214% to 4.9168%). For details see Figures 4b and 4B.

Correction for HCT > 45%

The results of Pasing-Bablok regression analysis showed that the slope between the CRP result from 7180 plasma and the result from the PA990pro when the HCT was fixed at 40% was 0.5997 (95% CI: 0.5325 to 0.6768, $p < 0.0001$). This suggests that there was a notable proportional systematic error between the two methods. The intercept was -2.4130 (95% CI: -4.7868 to 0.03914, $p = 0.0465$), suggesting that there was a constant systematic error between the two methods. For details see Figure 5a. The slope between the CRP re-

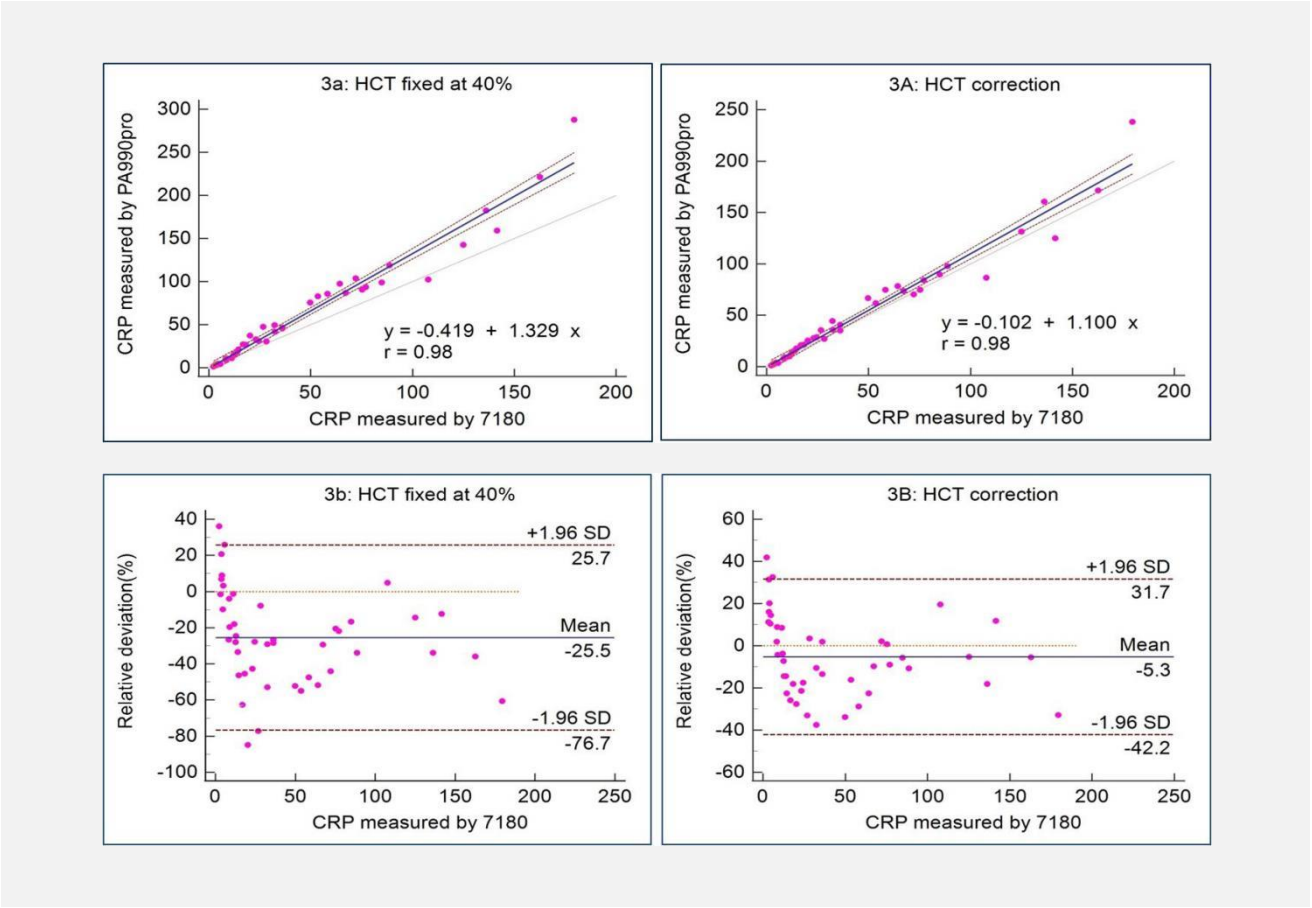

Figure 3. Comparison of CRP measured by the 7180 and CRP measured by the PA990pro (with or without HCT correction) when HCT < 35%.

Note: 3a and 3A are regression analysis figures of PA990pro and 7180 CRP detection. The solid line is the Passing-Bablok regression line, the dotted line on both sides is its 95%CI, and the fine solid line is Y = X. 3b and 3B show the relative deviation plots of the PA990pro and 7180 CRP detection. The relative deviation is calculated as CRP measured by PA990pro - CRP measured by 7180/CRP measured by 7180 (%), the solid line is the average relative deviation, and the upper and lower dashed line is ± 1.96SD.

sults from the 7180 plasma and the real-time HCT modified CRP test results from the PA990pro was 1.1283 (95% CI: 1.0460 to 1.2105, $p < 0.0001$). The intercept was -4.5469 (95% CI: -7.0777 to 2.0161, $p = 0.0008$). For details see Figure 5A. In summary, when the whole blood HCT was > 45%, the correlation between the results from 7180 plasma and PA990pro whole blood was poor when the HCT was fixed at 40% ($r < 0.975$). The correlation between the 7180 plasma and the PA990pro whole blood CRP tests, when corrected by HCT in real time, was good ($r > 0.975$). In addition, the real-time HCT correction of the PA990pro whole blood CRP results was able to correct the direction of error between the detection results of PA990pro (HCT fixed at 40%) and 7180 plasma.

Bland-Altman plots showed that compared with HCT fixed at 40%, the average relative deviation between real-time HCT corrected PA990pro whole blood CRP and 7180 plasma CRP decreased significantly from 54.7766% (95% CI: 47.7977% to 61.7554%) to 15.7566% (95% CI: 7.4534% to 24.0597%). For details see Figures 5b and 5B.

TG interference with whole blood CRP detection

High TG affected the CRP test results; the relative deviation of the CRP test results between samples with triglyceride concentrations below 7 mmol/L and samples with 0.9% normal saline were all within 10% (Table 2).

Bilirubin interference with whole blood CRP test results

High levels of bilirubin had little effect on the CRP results in this system; the relative deviations of CRP detection results between samples with different concentrations of bilirubin and samples without bilirubin were all within 10% (Table 3).

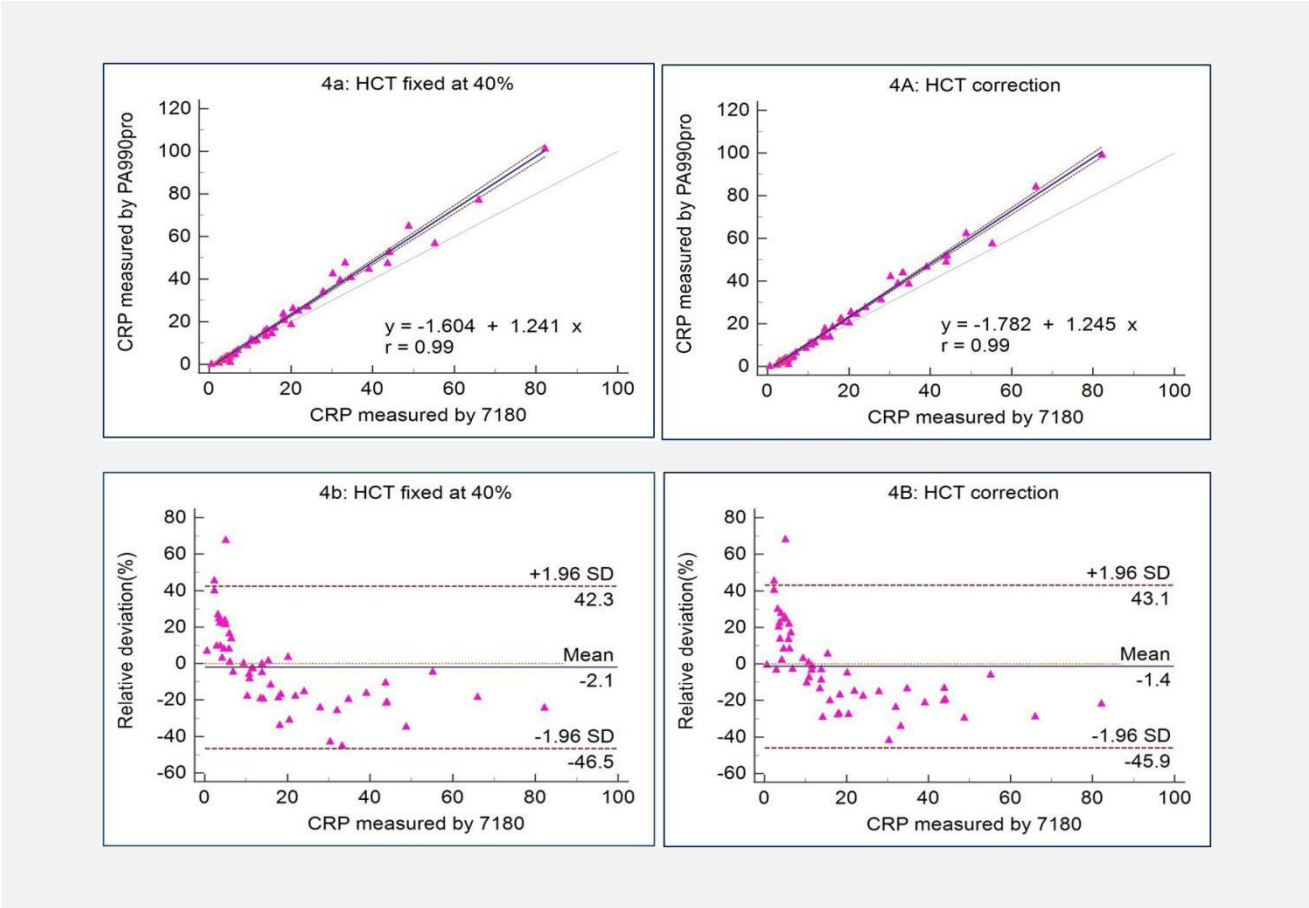

Figure 4. Comparison of 7180 plasma CRP and PA990pro whole blood CRP (with or without HCT correction) when HCT was 35% - 45%.

Note: 4a and 4A are regression analysis figures of the PA990pro and the 7180 CRP detection. The solid line is the Passing-Bablok regression line, the dotted line on both sides is its 95%CI, and the fine solid line is Y = X. 4b and 4B are relative deviation plots of the PA990pro and 7180 CRP detection, relative deviation which was calculated as CRP measured by PA990pro - CRP measured by 7180/CRP measured by 7180 (%), the solid line is the average relative deviation, and the upper and lower dashed lines are ± 1.96SD.

Correctness verification

The system passed the External Quality Assessment (EQA) by using the testing samples issued by the National Center for Clinical Laboratories (Table 4).

DISCUSSION

CRP was named for its ability to form a complex with *Streptococcus pneumoniae* capsular C polysaccharide in the presence of calcium ions [9]. Studies have confirmed that CRP detection can effectively reduce the use of antibiotics [10-12]. The cost of CRP detection is significantly lower than interleukin - 6 (IL - 6) and procalcitonin (PCT); as such, its use can effectively save medical and health costs [13]. In this study, the blank read value of whole blood CRP of the tested equipment was < 0.3 mg/L (the lower limit of detection of the kit was 0.3 mg/L), indicating that the ability of the tested equipment to quantify CRP was able to meet the clinical needs. Carryover contamination refers to the discontinuous amount of analyte carried from one test sample reaction to another by the measurement system, which erroneously affects the performance of subsequent test samples. In this study, the carryover contamination of whole blood CRP detection by the equipment was 0.05%, indicating that the high-value samples of whole blood CRP have little interference with the detection results of low-value samples of whole blood CRP. This finding meets the requirements of the manufacturer's instructions and the clinical needs. Factors that influence the repeatability of the test system can be divided into two categories. First, the level of automation built into the instrument itself factors; automated instrument systems are superior to semi-automatic instruments in terms of sampling volume accuracy of reagent and sample. Second, the influence of sample CRP concentration can influence the repeatability of the test. Generally

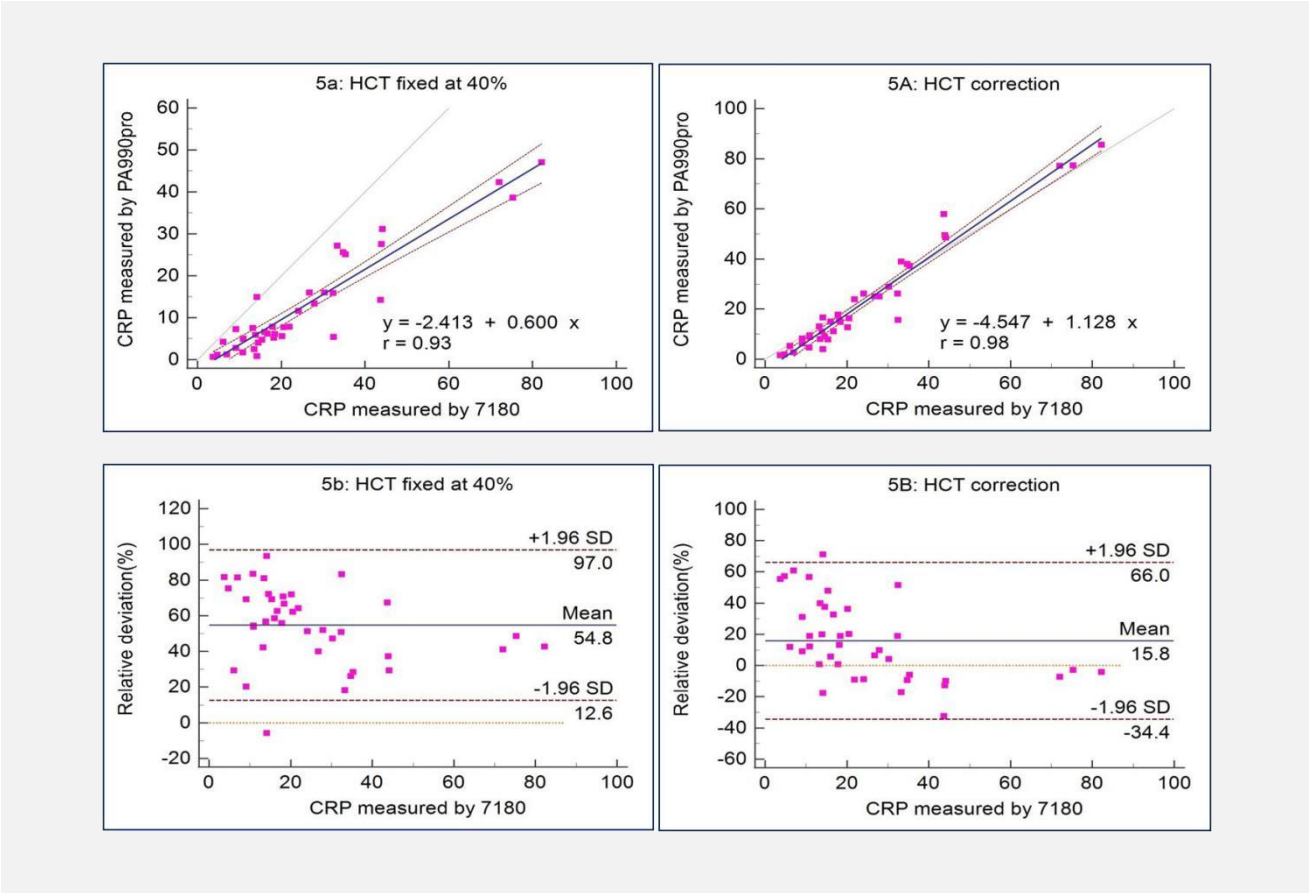

Figure 5. Comparison of CRP measured by the 7180 and CRP measured by the PA990pro (with or without HCT correction) when HCT > 45%.

Note: 5a and 5A are regression analysis figures from the PA990pro and the 7180 CRP detection. The solid line is the Passing-Bablok regression line, the dotted line on both sides is its 95%CI, and the fine solid line is Y = X. 5b and 5B are the relative deviation plots from the PA990pro and the 7180 CRP detection; the relative deviation is calculated as CRP measured by PA990pro - CRP measured by 7180/CRP measured by 7180 (%), the solid line is the average relative deviation, and the upper and lower dashed lines are ± 1.96SD.

speaking, the CV of low concentration samples was larger, and those of medium and high concentration samples were smaller. CVs for repeatability and intermediate precision were consistent with Scharnhorst's report [14].

Due to the wide linear range of CRP, the linearity evaluation of this study was carried out in three sections. Mixing of high and low concentration whole blood CRP samples from different clinical sources can be imprecise in terms of consistently generating the target HCT. In addition, hemolysis may occur due to blood group incompatibility. Therefore, in this study, serum samples were used to prepare various CRP sample concentrations for linear analysis. We found that the linear range of CRP detection by this instrument was good (slope was between 0.950 and 1.050 and the r-value was > 0.975), which may meet the clinical criteria.

Sample stability refers to the ability of specimens stored under specified conditions to maintain the consistency of measured values within a specified time and a specific limit value. The testing of blood samples may be delayed due to laboratory or clinical reasons; thus, it was necessary to test how sample storage conditions and storage time affect test results. The sample stability experiment in this study showed that the CRP test results of whole blood could remain stable within 72 hours at room temperature (18 - 25°C) or under refrigeration (2 - 8°C); the relative deviation was < 10%, which could meet the clinical needs. Our findings paralleled those of Juan Cheng et al. [15].

Triglyceride and bilirubin are common interference factors in laboratory testing. The interference experiments in this study showed that the interference bias caused by bilirubin (< 216 μmol/L) to the system was less than 10%, which could meet the clinical needs. The interference bias caused by triglyceride (< 7 mmol/L) to the system was < 10%. Therefore, high concentration triglycerides can affect CRP results, especially in CRP samples with low concentrations, as can be seen in Table 2.

CRP is a plasma protein [9] that is mainly present in the plasma or serum of blood samples. In clinical practice, CRP concentration refers to serum or plasma. To correspond with present clinical practice, whole blood sample test results needed to be converted to obtain the serum or plasma CRP concentration of the specimen. In the detection of whole blood CRP, the instrument draws a certain sample volume. Depending on the hematocrit, the plasma proportion in a fixed volume of a whole blood sample will differ. This affects the accuracy of whole blood CRP detection. However, the Lifotronic PA990pro has no HCT measurement correction function. We found that the results of whole blood CRP detection at various dilution concentration points of HCT were negatively correlated with the results of whole blood CRP detection at 40% dilution concentration points (see Table 1). The largest interference factor of whole blood CRP detection by this instrument comes from HCT. For the whole blood CRP detection equipment without any HCT measurement function, the HCT is generally set to a fixed value. When the actual HCT is higher than this fixed value, the test result is lower than the real value. When the actual HCT is lower than this fixed value, the test result is inflated (see Table 1 for details). In the clinical sample verification, for the samples with whole blood HCT < 35%, real-time correction of HCT could effectively reduce the average relative deviation of the CRP test results between the PA990pro and the 7180 analyzer. In addition, for samples with whole blood HCT > 45%, real-time correction of whole blood CRP detection results could correct the direction of deviation from the 7180 plasma CRP detection results, and could significantly improve the correlation between the two methods (when HCT was fixed at 40%, r = 0.93; after real-time correction of HCT, r = 0.98 between the two methods). It is worth noting that across the range of HCTs, the correlation between the results of HCT real-time corrected whole blood CRP detection and the results of the 7180 plasma CRP detection was good (r > 0.975).

At present, rapid whole blood CRP tests are mainly used in outpatient, emergency, and pediatric settings when high turn-around time (TAT) timeliness requirements allow. Clinically, individuals with a large amount of body fluid and blood loss, polycythemia vera, and neonates can show an increase in hematocrit. Anemia, itself caused by various factors, can lead to a decrease in hematocrit. The above clinical conditions can affect the accuracy of the whole blood CRP measurement results. Therefore, attention must be paid to the influence of HCT on CRP results. In clinical practice, whole blood CRP detection is usually performed at the same time as blood cell analysis for rapid differential diagnosis of bacterial or viral infections. The PA990pro is usually used in combination with a Sysmex hematology analyzer in the assembly line. Therefore, although the PA990pro has no HCT measurement function, the concentration of PA990pro whole blood CRP can be corrected by testing for HCT in the hematology analyzer through the intermediate software system. However, it should be noted that the accuracy of whole blood CRP detection results in patients with abnormally high and low HCT values should be addressed by testing institutions that use PA990pro alone. It is suggested to utilize the HCT results of blood cell analysis of the patient during the same period through the laboratory information system (LIS), and set the CRP correction formula on the LIS system ($CRP_{corrected} = CRP_{measured}*(1 - 40\%)/(1 - HCT_{measured})$). This approach offers a simple, rapid, and cost-free method of obtaining modified whole blood CRP test results that can meet clinical needs.

Finally, the correctness of the PA990pro CRP was verified by using external quality assessment samples from National Center for Clinical Laboratories, and all passed the assessment.

This study found that the CRP detection performance of the Lifotronic PA990pro specific protein analyzer can meet clinical requirements. However, in clinical application, attention should be paid to the impact of abnormal HCT on the test results. It is suggested that the LIS system offers an amended formula to ensure the accuracy and reliability of the whole blood CRP test.

Acknowledgment:
The authors would like to express their gratitude to EditSprings (https://www.editsprings.cn) for the expert linguistic services provided.

Source of Funds:
Beijing JST Research Funding [Grant No. QN202217].

Declaration of Interest:
None.

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