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ORIGINAL ARTICLE

Immunoturbidimetric Assay for Determination of Peripheral Blood C Reactive Protein on the Pentra MS CRP Hematology Analyzer

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SUMMARY

Background: **The Pentra MS CRP hematology analyzer (hereinafter the Pentra analyzer) can simultaneously provide 5-part leukocyte differential and C-reactive protein (CRP). The aim of the study was to investigate the performance of CRP determination by the Pentra analyzer.**
Methods: **The precision, limit of quantitation (LoQ), carryover, linearity, stability, and comparability of the Pentra analyzer were determined. The Passing-Bablok regression analysis and the Bland-Altman graphs illustrated the correlation for CRP concentration analyzed by the Pentra analyzer and BN-II analyzer.**
Results: **The within-run precision of CRP determination by the Pentra analyzer had a CV < 2.0% in peripheral blood, which met the requirements of the instructions (CV ≤ 10%). The Pentra analyzer had a total CV of 5.35% and 5.52% at a CRP concentration of 4.1 and 80 mg/L, respectively. The LoQ value for the Pentra analyzer was 0.96 mg/L. The carryover was 0.57% for peripheral blood and 0.86% for plasma by the analyzer. The stability of CRP results was good, when the anticoagulation samples were stored at room temperature or 4°C within 48 hours (deviation < 5%). The linearity range for whole blood samples was 0 - 188.13 mg/L (r^2 = 0.9992). There was high correlation of the CRP results analyzed with the Pentra analyzer and BN II analyzer. The Passing-Bablok regression analysis and the Bland-Altman graphs showed the bias plot display excellent agreement between the two assays (the mean value for the Pentra 2.19 mg/L and the BN-II 2.35 mg/L, n = 101).**
Conclusions: **The results of CRP determination by the Pentra analyzer have the advantages of accuracy and reliability, and it is suitable for routine use in emergency laboratory and small to medium-size laboratories.**
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KEY WORDS

Pentra MS CRP hematology analyser, C-reactive protein, peripheral blood

INTRODUCTION

C-reactive protein (CRP) is one of the acute-phase proteins. The serum or plasma levels rise during general, nonspecific response to infectious and non-infectious inflammatory processes [1]. CRP was also shown to be a useful and accurate screening test for bacterial infections, showing a sensitivity of 0.69 (0.53 - 0.60), specificity of 0.76 [2]. CRP is often used to diagnose a varie-

ty of inflammatory diseases, extensive trauma, burns, surgery, and acute myocardial infarction [3,4].

In various disease states resulting in tissue injury, infection or acute inflammation, CRP values may rise above normal to 20 - 500 mg/L [5]. As elevated CRP values are always associated with pathological changes, the CRP assay provides useful information for the diagnosis, therapy, and monitoring of inflammatory processes and associated diseases. Studies have shown that measurement of CRP by high sensitivity assays is a strong independent predictor of risk of future cardiovascular and peripheral vascular disease [6].

Over the last decades, various CRP assays have been developed, including the enzyme-linked immunosorbent assay (ELISA), immunoturbidimetry, time-resolved immune-fluorimetry assay, and mass spectrometry [7-10]. Among the assays, immunoturbidimetry assays possess greater precision at low concentrations of CRP and automated assays have been developed that are suitable for routine use in the clinical laboratory.

CRP can be measured qualitatively, semi-quantitatively, and quantitatively. The qualitative method measures the absence or presence of a certain level of CRP in a serum sample [11]. The semi-quantitative method measures an approximate concentration of CRP within a serum sample. The quantitative method is more complex and expensive, requiring more time to perform (15 - 30 minutes) [12].

Although CRP appears to be a useful screening test for various inflammatory diseases, it was advised that CRP should not be used as a single marker to diagnose sepsis but rather should be associated with clinical evaluations and other tests. A complete blood cell count (CBC) including leukocyte differentiation and C-reactive protein (CRP) are frequently examined as laboratory markers for patients with suspicious inflammatory diseases such as acute bacterial infection [13,14]. Therefore, an automated hematology analyzer that is capable of simultaneously and rapidly measuring CBC and CRP is of significant value in various clinical settings.

The Pentra MS CRP hematology analyzer (ABX, Horiba, Ltd., hereinafter the Pentra analyzer) has been designed to resolve this clinically important problem. There were several studies recorded for the automated hematology analyzer. Therefore, in the present study, we mainly evaluated the CRP performance analyzed by the Pentra analyzer and its correlations with the Siemens Behring Nephelometer II analyzer (Dade Behring, Marburg, Germany) to validate its acceptability in clinical settings.

MATERIALS AND METHODS

Samples

The samples used for the evaluation came from the outpatient and admission departments at the Chinese People's Liberation Army General Hospital. The samples were collected and anticoagulated with EDTA-K_2. Specimens with hemolysis, blood coagulation, or clots were ruled out according to the Collection of Diagnostic Venous Blood Specimens [15-17]. Whole blood samples were used to evaluate the CRP analytic performance of the Pentra analyzer. The peripheral blood samples were then centrifuged at 2,400 g at room temperature for 10 minutes, and the supernatants were further used to measure CRP with a latex immunonephelometric assay on a Siemens Behring Nephelometer II analyzer (Dade Behring, Marburg, Germany).

The study was approved by the Institutional Ethics Committee of the Chinese People's Liberation Army General Hospital, and all participants gave their informed consent before inclusion in this study.

Analytical principle for CRP on the Pentra analyzer

The assay was a 2-reagent system and certificated by the China Food and Drug Administration (no. 20162 400581). The kit consists of a Lyze reagent 1 (code no. 12-0910P), assay buffer reagent 2 (code no. 12-0910P), rabbit anti-CRP immunoparticles reagent 3 (code no. 12-0910P), calibrator (LOT, 180724K3), with concentration of 40 mg/dL, and two controls (code No. 12-1221P). Measurements were performed on the Pentra analyzer, situated at the Chinese People's Liberation Army General Hospital, Beijing. The CRP assay is based on a turbidimetric immunoassay technique. The anti-CRP-immunoparticles aggregate with human CRP and form complexes that can be measured with turbidimetric methods and are correlated with human CRP concentrations by interpolation on a calibration curve. The assay buffer is used to enhance the reaction and to prevent unspecific interactions. The following assay protocol was used in all experiments: 100 µL reagent 1 and 8 µL sample were mixed and incubated for 1 minute at 37°C, 50 µL reagent 2 were added and incubated for 20 seconds at 37°C, and 50 µL reagent 3 were added and incubated for 20 seconds at 37°C. Then the immune complex formed caused an agglutination of the antibody coated particles. The absorbance measured the agglutination reaction at wavelength 660 nm. The total assay time was 3.5 minutes and a non-linearity mode calibration method was used for evaluation. Subsequently, each obtained value of the hemolyzed sample is converted to plasma concentration according to the hematocrit (HCT; %) of the respective sample to obtain a value for 'whole blood CRP'. The CRP computational formula is F (CRP sensitivity factor) x calibration coefficient x HCT. The specimen should be diluted and reanalyzed when the result shows "--, --D".

Assay imprecision

The assay imprecision was evaluated following the National Committee for Clinical Laboratory Standards (NCCLS) protocol EP5-A2 [18] using two replicates per day for two CRP levels of control material (4.1 and 80 mg/L) over 20 days (n = 40 per control level). The intra-assay, the inter-assay, and the total assay variations were calculated.

Limit of quantitation
The limit of quantitation (LoQ) was determined from repeat analysis of the two lowest standards and serial dilutions of the lowest standard for each CRP assay, on five separate days. LoQ is defined as the concentration that results in a CV = 20% (or some other predetermined % CV) and is thus a measure of an assay's precision at low analyte levels (without addressing bias) [19].
To check linearity, the calibrator was linearly diluted using human CRP standard product ERM-DA474/ IFCC, yielding samples containing 100%, 80%, 60%, 40%, 20%, and 0% of the actual calibrator concentration of 41.2 mg/L CRP.

Carryover
Carryover for CRP was calculated according to ICSH guidelines H26-A2c [20]. A high concentration of sample was measured consecutively on the Pentra three times (H1, H2, H3), followed immediately by a diluent consecutively for three times (L1, L2, L3). Carryover was calculated by the following formula: Carryover (%) = (L1 - L3)/(H3 - L3) x 100.

Linearity
Linearity was evaluated for CRP according to CLSI EP6-A [21]. Six dilutions of fresh patient samples were made in plasma (0%, 10%, 20%, 40%, 60%, 80%, and 100%) and performed in random order for three times when the carryover passed, then the average of the replicates for each concentration was taken to check the linearity. The results were compared with ideal values and correlation coefficients. A regression equation was calculated for CRP at each dilution. CRP specimens were manually prepared using 226 mg/L concentrated CRP serum (concentration identified by Behring Nephelometer II analyzer) and saline water.

Interference effect for the CRP
Interferences were studied by mixing 1 part of interfering material with 9 parts of serum. Various concentrations of the following substances were tested for interference: hemoglobin (50, 40, 30, 20, and 10 g/L), bilirubin (0.3, 0.2, 0.15, 0.1, and 0.5 g/L), and intralipid (2.0%, 1.5%, 1.0%, and 0.5%). These samples were subsequently examined according to CLSI EP7-A2 [22]. A measured CRP concentration of between 85% and 115% of the expected concentration was considered as acceptable.

Sample stability
Five fresh samples with CRP concentrations ranging over the whole calibration series were collected, aliquoted, and stored at room temperature and 4°C for up to 72 hours. The samples were analyzed initially and reanalyzed in duplicates after 1, 6, 12, 24, 48, and 72 hours of storage. Change in CRP concentrations was expressed as the percentage of the initial concentration.

Method comparison
To conduct a relevant study of the CRP results provided by the Pentra and BN-II analyzers, 101 samples were randomly collected from patients in the intensive care and inpatient department of the Chinese PLA general hospital. Measurement procedures were compared by linear regression analysis according to the nonparametric Passing and Bablok method [23] and by analysis of differences according to Bland and Altman [24].

Statistical analysis
Statistical analyses were performed utilizing SPSS 22.0 (SPSS Inc., IBM Corp., Armonk, NY, USA) and the MedCalc software (version 11.4.2). Data were presented as mean ± SD. Differences between groups were established by unpaired *t*-test for normally distributed values and by Kruskal-Wallis analysis followed by Dunn's test for nonparametric values. The distribution was checked against normal distribution to verify that the transformed material showed normal distribution. Agreements between the Pentra analyzer and the BN II analyzer were assessed by the method of Bland and Altman. Results were considered statistically significant at $p < 0.05$.

RESULTS

Analytical performance
Within and between run variations and total assay imprecision were calculated using control samples at two levels of CRP. The Pentra assay had a total CV of 5.35% and 5.52% at a CRP concentration of 4.1 and 80 mg/L, respectively. The imprecision data were shown in Table 1. The LoQ value (including any sample dilution factors where relevant) for the Pentra assay was 0.96 mg/L. The carryover rates were 0.57% for anticoagulation peripheral blood and 0.86% for plasma, which were all < 2.0% as required by manufacturers. Data from a linearity study depicted that the CRP assay was linear ($r^2 = 0.9992$) and the studied range of 0 - 226 mg/L and the regression equation between the expected and the observed CRP value was $y = 1.032x - 1.179$ (Figure 1).
Regarding the interference effect for the CRP study, the hemoglobin (up to 50 g/L), bilirubin (up to 0.3 g/L), and intralipid (up to 5%) did not show any significant interference.
Five whole blood samples were stored at room temperature and refrigerator temperature for up to 72 hours. The samples were fairly stable at the two different temperatures and changes in concentrations were below 5% after 48 hours and below 6% after 72 hours storage in refrigerator.

Method comparison
The Passing-Bablok regression analysis and the Bland-Altman graphs were illustrated in Figure 2 and 3. The regression analysis showed that an excellent correlation

Table 1. The imprecision of CRP measured by Pentra.

Precision estimates	Level 1	Level 2
Mean concentration (mg/L)	4.10 ± 0.067	80 ± 1.421
Within run CV (%)	1.63	1.9
Between run CV (%)	7.22	4.45
Total assay imprecision	5.35	5.52

Table 2. The LoQ for CRP measured by the Pentra.

CRP (mg/L)	Sample		
	1	2	3
Concentration	0.96	2.49	4.1
SD	0.18	0.27	0.4
CV%	18.85	10.7	9.66

Table 3. The carryover measured on the Pentra hematological analyser.

Sample type	Concentration (mg/L)						Carryover (%)	Manufacturer's specifications (%)
	H1	H2	H3	L1	L2	L3		
Whole blood	162	185	181	6	5	5	0.57	< 2.0
Serum	126.1	131.6	131.8	16.4	15.5	15.4	0.86	< 2.0

Table 4. Change of CRP concentrations with different temperature.

Hours	23 ± 2°C		4 ± 2°C	
	Mean	Deviation (%)	Mean	Deviation (%)
1	47.02	-	47.02	-
6	47.83	1.72	48.16	2.42
12	47.44	0.89	48.51	3.17
24	46.22	-1.7	46.88	-0.3
48	46.26	-1.62	49.34	4.93
72	45.81	-2.57	49.81	5.93

and satisfying agreement between both methods, as indicated by the slope (1.01; 95% CI, 0.96 - 1.00) and intercept (0.015; 95% CI, 0.01 - 0.027). Spearman's rank correlation coefficient was r = 0.990 ($p < 0.001$). The bias plot displayed excellent agreement between the two assays (the mean value for Pentra 2.19 mg/L and BN II 2.35 mg/L).

DISCUSSION

CRP is an acute-phase reactant, whose plasma concentration increases during inflammatory disorders [25, 26]. CRP consists of five identical subunits arranged as a cyclic pentamer. CRP has long been recognized as a useful marker and gauge of inflammation. It is also valuable for assessing the severity of inflammatory dis-

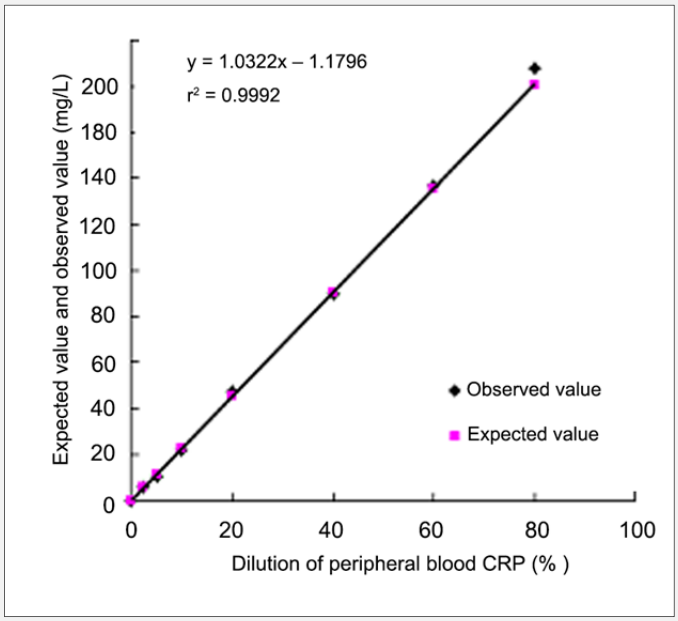

Figure 1. Linearity study depicted the CRP assay was linear (r^2 = 0.9992).

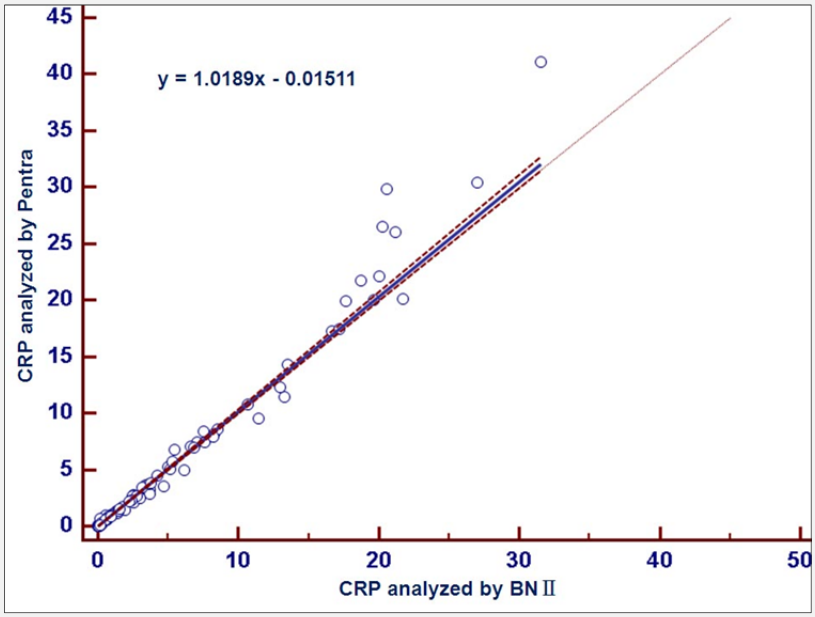

Figure 2. The Passing-Bablok regression analysis on the CRP analyzed by the Pentra analyzer and the BN II analyzers (n = 101).

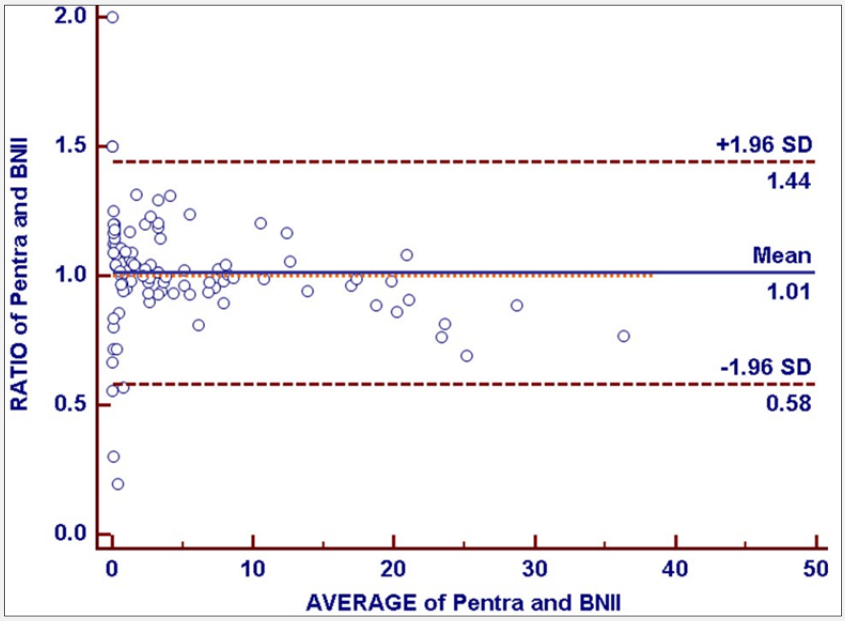

Figure 3. The Bland-Altman graph analysis of the CRP analyzed by the Pentra analyzer and the BN II analyzer (n = 101).

orders and as a prognostic tool in the follow-up of response to treatments [27].

In present study, the data showed that the within-run precision of the measurement of CRP by Pentra: CV < 5.4% of anticoagulant peripheral blood and CV < 2.0% of quality control substance of CRP, all met the requirements of the instrument operating instructions (CV < 10%). The carryover was 0.57% for the anticoagulant in peripheral blood, which was lower than the requirement of manufacturers of < 2.0%. The results of CRP were stable and the relative deviation was < 5.0% within 48 hours after preservation of anticoagulant samples at room temperature or 4°C.

The peripheral blood CRP was in the range of 0 mg/L ~ 188.3 mg/L, and the measured value was consistent with the theoretical value, showing good linearity. When Pentra determines CRP without displaying the value and only displays "-- -d", the specimen should be diluted according to the instrument operation manual and then re-analyzed CRP to ensure an accurate result. The LoQ of CRP measurement in peripheral blood was 1 mg/L, which was less than the limit of 2 mg/L as described in the operating manual of the instrument to be evaluated.

By analyzing the hemolytic specimens and the simulated clinical lipidemic specimens with a certain amount of medical fat milk, it was found that the hemolytic or lipidemic specimens in the common clinical range did not significantly interfere with the CRP results measured by the Pentra.

When peripheral blood samples were used to analyze CRP, different hematocrit directly leads to the different CRP results for the plasma proportions. Some factors can affect the accuracy of the peripheral blood CRP results. These factors include blood or body fluid loss, polycythemia vera disease, etc. Therefore, it is recommended peripheral blood CRP be analyzed according to hematocrit and correction formula. These factors were taken into account in the design of the Pentra analyzer, and the CRP concentration result was calculated automatically by the formula "CRP = F (CRP curve, CRP reagent factor) x calibration coefficient x HCT coefficient", which ensured the accuracy of CRP results in peripheral blood.

The rapid increase of serum CRP is related to the involvement of inflammation, tissue injury, and repair process. However, it is very difficult to collect 3 mL blood to analyze CRP concentration from infants by the traditional method. It is very rapid and convenient for the Pentra analyzer to detect the CRP concentration. The analyzer has been designed to simultaneously analyze CBC and CRP within 3.5 minutes only needing 35 µL peripheral blood. CRP and WBC levels are of high diagnostic values especially to neonatal infectious diseases [28]. The Pentra analyzer is helpful for the diagnosis in neonatal infectious diseases by analyzing CRP.

The Pentra analyzer, an instrument that can simulta-

neously do a complete blood count (CBC) and white blood cell differentiation (DC), has two modes of DIFF (CBC + DC) and DIFF + CRP, which can be switched at will, and CBC, DC, and CRP results can be reported in 3.5 minutes, which facilitates clinical diagnosis and treatment.

CONCLUSION

With the advantages of convenience and easy use, the Pentra analyzer is suitable for routine use in emergency laboratory and small to medium-size laboratories.

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Declaration of Interest:
None.

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