

FULL PAPER

Agreement between whole-blood CRP measurement using Humacount 5DCRP and serum CRP measurement by immunoturbidimetry (Abbott Alinity CI Series): A comparative study

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C-reactive protein (CRP), an acute-phase protein in response to infection, inflammation, and tissue damage, is useful for both diagnosis and monitoring. Nowadays, serum CRP with immunoturbidimetry is a popular method for measuring CRP. This method requires more time for preparation and larger sample volumes. Humacount 5D^{CRP}, a hematology analyzer, offers faster, single-run Complete Blood Count and CRP tests, reducing turn-around-time (TAT). However, this new method should be validated and compared with the established method. The objective of this study was to evaluate the agreement between whole-blood Humacount 5D^{CRP} and serum CRP level and immunoturbidimetry method (Abbott Alinity). The CRP levels of 65 patients were each examined using whole-blood samples in Humacount 5D^{CRP} and serum samples in Abbott Alinity. The correlation of CRP values was evaluated using Spearman test. The reliability test used Cohen Kappa agreement coefficient. Bland-Altman curve test analysis was used to examine the difference in limit of agreement of CRP levels between those analyzers. Spearman test showed a very strong positive correlation ($R=0.956$, $p<0.001$). Kappa agreement test analysis revealed almost perfect agreement ($R = 0.938$, $p < 0.001$). Bland-Altman curve analysis showed a mean difference of -2.59 (95% CI $-12.34 - 7.16$), where of the 65 samples tested, only four samples had a difference in CRP levels outside the limit of agreement value. The whole blood CRP with Humacount 5D^{CRP} is strongly agreed with CRP with Abbott Alinity. It can serve as a reliable CRP testing, provide rapid results to support faster clinical decision-making, and improve patient management.

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KEYWORDSC-reactive protein; immunoturbidimetry; hematology analyzer; Humacount 5D^{CRP}; Abbott Alinity CI series.**Introduction**

C-reactive protein (CRP) is an acute-phase protein generated by hepatocytes. Structurally, CRP is a pentamer consisting of

five identical subunits bound by non-covalent bonds [1]. The CRP synthesis by hepatocytes is mainly regulated by IL-6, a pro-inflammatory cytokine, which is then secreted into the plasma. The CRP can increase up to

1,000 times in conditions related to infection, inflammation, and tissue injury. In severe bacterial infections and burns, CRP levels can increase to over 20 mg/dL, whereas in viral infections, they typically range between 1 and 4 mg/dL [2].

CRP levels begin to increase 4-10 hours after an inflammatory response and peak within 48 hours. CRP elevation can be detected even before clinical symptoms appear or an increase of leukocytes in infection. After the triggering factor subsides, CRP concentrations decrease exponentially within 18-20 hours, which aligns with its half-life. In cases of severe tissue injury, such as trauma or advanced cancer, CRP levels can surge from approximately 0.1 mg/dL to more than 50 mg/dL within 24-72 hours [3]. These traits render it very suitable for monitoring therapy and predicting the outcome of a disease [4,5].

The analytical method for detecting CRP levels must have several characteristics, such as minimal sample volume, good sensitivity and specificity, short examination time, and accurate results [6]. There are more than ten CRP testing methods; nevertheless, immunoassay techniques are the most widely used [1]. Several *in vitro* techniques, including turbidimetric assays (immunotransmission turbidity and immunosorbent turbidity), enzyme-linked immunosorbent assay (ELISA), alongside chemiluminescent, fluorescent, lateral flow assays (LFAs), and electrochemical assays are available. These methods vary in their detection limits: turbidimetric assays typically measure CRP at the $\mu\text{g/mL}$, whereas ELISA, chemiluminescent, fluorescent, and electrochemical techniques can detect CRP at much lower concentrations, down to the fg/mL [7]. Differences in detection systems, reagents, and methodological principles can lead to variations in CRP measurement results. Since healthcare facilities often use different CRP testing instruments, ensuring consistency across various testing methods is essential. In

addition, the instrument should be user-friendly and widely applicable in clinical settings [6].

While CRP testing using serum or plasma samples through immunoturbidimetric methods is widely accepted as the gold standard, these methods have limitations, particularly regarding the need for relatively large sample volumes and longer processing times [8,9]. Furthermore, these factors can be challenging in emergency conditions and outpatient clinics that need fast results or patients with difficult venous access, such as neonates or critically ill patients [5]. Conducting CRP tests with whole-blood samples would overcome this problem. It has the potential to decrease both the processing time of the samples as well as their volume [9].

Previous studies have demonstrated a strong correlation between whole blood CRP and serum CRP tests conducted on various instruments. In 2014, Woolley evaluated the correlation between whole-blood CRP levels measured by Microsemi CRP and serum CRP levels measured by Cobas 6000, reporting a good correlation ($R^2 = 0.996$) [10]. A study by Nomura assessed the correlation between whole-blood CRP levels measured by Microsemi CRP and serum CRP levels measured by Hitachi 7600. This study demonstrated a strong correlation with $r \geq 0.997$ [9]. A different study compared the efficacy of three analyzers, Mindray BC 5390, Mindray BC 6800 (using whole-blood samples), and Johnson Vitros 5600 (using serum samples), for CRP measurement. The data showed a positive correlation across all three analyzers; however, Johnson Vitros 5600 required a larger sample volume, took longer examination time, and was more expensive than Mindray BC 5390 and Mindray BC 6800 [6].

Humacount 5D^{CRP} is a hematology analyzer that includes an extra CRP test conducted via the immunoturbidimetry technique. This analyzer only requires one EDTA blood

sample (whole-blood) for the Complete Blood Test and CRP test, thus requiring minimal blood volume. Moreover, Humacount 5D^{CRP} also offers an option for capillary blood samples, making it very helpful in situations where blood samples are difficult to obtain [11]. Nowadays, there are not many hematology analyzers on the market today that have the option of CRP test from whole blood samples.

Although previous studies have shown a positive correlation between whole blood CRP and serum CRP measurement for different devices, its accuracy and consistency with the well-established immunoturbidimetry method, as used in Abbott Alinity, remain unclear and it should be validated [12]. Since Abbott Alinity is frequently utilized in Dr Soetomo General Hospital for CRP testing, it is crucial to validate Humacount 5D^{CRP} against this reference method in order to determine its potential for broader clinical applications. This study aims to address this gap by evaluating the agreement between CRP measurements from whole-blood samples via Humacount 5D^{CRP} and serum CRP values measured using the immunoturbidimetry method (Abbott Alinity CI series, Abbott, Illinois, U.S.A.), and ultimately wanted to prove whether the whole blood CRP test could serve as a reliable and efficient alternative to conventional serum-based methods.

Materials and methods

This study was a cross-sectional and observational study. The data were consecutively collected. This study was performed in the Central Laboratory of the Clinical Pathology Unit of Dr Soetomo General Hospital from July 15th, 2023 to January 15th, 2024. This study recruited 65 men and women who underwent complete blood count and CRP at the Clinical Pathology Laboratory Unit of Dr Soetomo General Hospital Surabaya.

The inclusion criteria were patients who were more than 18 years old and underwent complete blood count and CRP at the Clinical Pathology Laboratory Unit of Dr. Soetomo General Hospital Surabaya. The exclusion criteria were clotted samples, hemolyzed samples, or samples that could not be detected by the instruments due to preanalytical error.

Laboratory data measurement

The CRP levels of each patient were performed from whole-blood samples and serum samples. CRP examination using whole-blood samples was performed with Humacount 5D^{CRP}, while CRP using serum samples was yielded with Abbott Alinity CI Series. Whole-blood samples were collected from vein puncture and kept in 3 mL EDTA tubes. Serum samples were collected from vein puncture and kept in a 5 mL SST tube. In addition, the SST tubes were incubated for 30 minutes before being centrifuged at 4400 rpm for 15 minutes. All samples were kept at ambient temperature, and the CRP measurement was performed in less than 24 hours.

Ethical consideration and informed consent

The study was approved by the Committee of Research and Ethics in Dr. Soetomo General Hospital (Surabaya, Indonesia) with approval number 2253/121/3/VI/2023. All procedures followed ethical guidelines, and informed consent was obtained from all participants before sample collection.

Statistical analysis

The demographic data were descriptively presented. The data of the study included the CRP levels either from the serum samples tested with Abbott Alinity C series or from the whole-blood samples analyzed with Humacount 5D^{CRP} analyzer, presented as Mean \pm SD. The normality test was performed with

the Kolmogorov-Smirnov test. The correlation between CRP levels from both instruments was analyzed with the Spearman correlation due to the normality of the data. The reliability of both CRP levels was performed with Cohen's Kappa coefficient. Agreement analysis was assessed with Bland-Altman analysis. The p-value less than 0.05 was considered statistically significant.

Results

This study analyzed data from 65 patient samples, which included 32 males and 33 females over the age of 18. CRP levels measured using the Humacount 5DCRP varied from 0.10 mg/dL to 18.69 mg/dL, with an average \pm standard deviation (SD) of 6.11 ± 5.78 mg/dL. Meanwhile, CRP levels measured using the Abbott Alinity CI series showed a minimum of 0.10 mg/dL and a maximum of 42.25 mg/dL, with a mean \pm SD of 8.70 ± 9.64 mg/dL as shown in Figure 1. The Mann-Whitney showed there is no significant difference between groups ($p = 0.31$).

Normality test of CRP data

To assess data distribution, the Kolmogorov-Smirnov test was performed. The test yielded a p-value of <0.001 , indicating that the data

were not normally distributed. Therefore, a correlation analysis was conducted using Spearman's correlation test.

Correlation analysis between humacount 5DCRP and Abbott Alinity CI Series CRP values

The Spearman correlation analysis between CRP values from the Humacount 5DCRP and the Abbott Alinity CI series produced a correlation coefficient (r) of 0.956 ($P < 0.001$). These results signify a very strong positive correlation, as demonstrated in the scatterplot (Figure 2).

Agreement analysis of CRP values between humacount 5DCRP and Abbott Alinity CI Series

A kappa test was performed to evaluate agreement between the two analyzers by categorizing CRP values into high and low levels based on the median of each instrument. Sixty-five samples were tested, 63 of them showed consistent results, while two others showed discrepancies, as indicated in Table 1. The agreement analysis yielded a kappa coefficient (r) of 0.938 ($P < 0.001$), indicating almost perfect agreement between the two measurement methods.

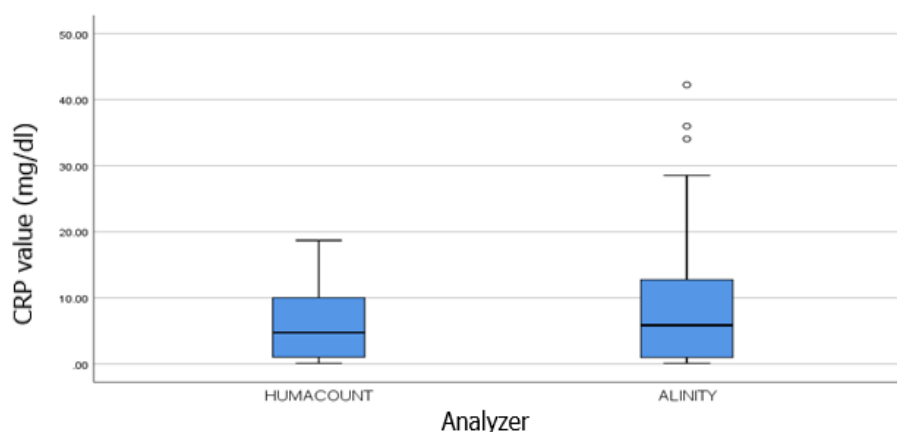


FIGURE 1 Descriptive analysis of CRP values on Humacount 5DCRP and Abbott Alinity CI series

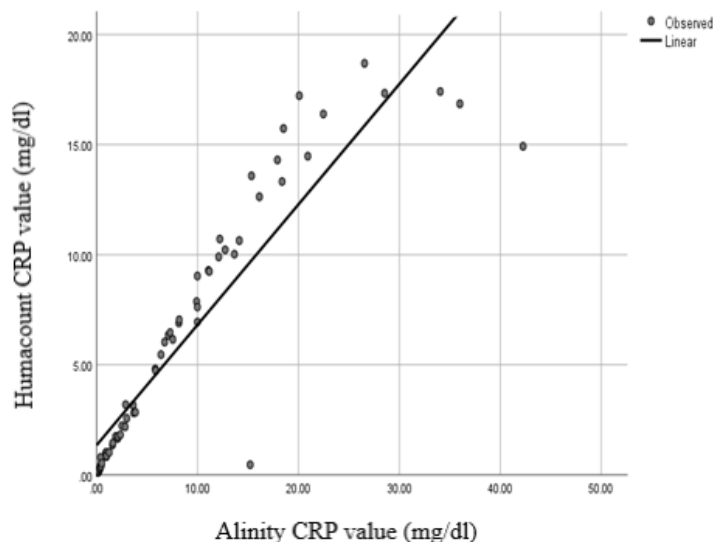


FIGURE 2 Scatterplot of CRP level correlation between Humacount 5D^{CRP} and Abbott Alinity CI series

TABLE 1 Kappa agreement test between Humacount 5D^{CRP} and Abbott Alinity CI series

CRP value	Humacount		p-value	r Kappa
	Low	High		
Alinity				
Low	31	1	< 0.001	0.938
High	1	32		

Bland-Altman Analysis of CRP Values between Humacount 5D^{CRP} and Abbott Alinity CI Series

The Bland-Altman analysis (Figure 3) showed a mean difference of -2.59, suggesting that the Humacount 5D^{CRP} tended to provide slightly

lower CRP readings than the Abbott Alinity CI Series, as demonstrated in Figure 3. The 95% confidence interval for the difference ranged from -12.34 to 7.16. Only four samples out of 65 had CRP values outside the limits of agreement.

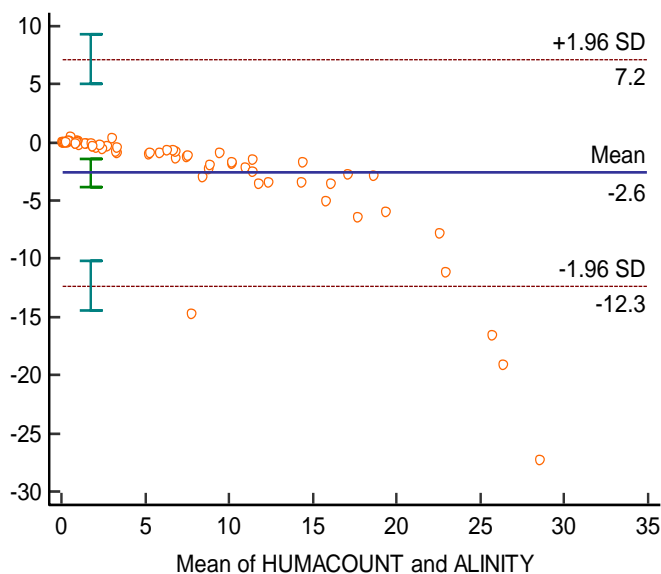


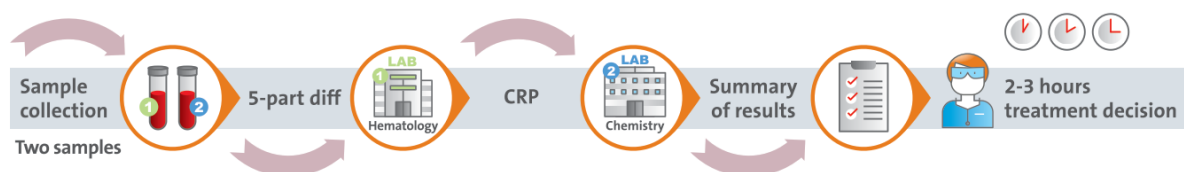
FIGURE 3 Bland-Altman plot of CRP levels between Humacount 5D^{CRP} and Abbott Alinity CI series

Discussion

Complete blood count (CBC) testing with whole-blood samples is one of the most frequently requested tests by clinicians. The recent developments in haematology analyzers have enabled CBC and CRP to be performed simultaneously on the same instrument using whole-blood samples. These advancements were more efficient in terms of

both cost and time [5,6]. The Humacount 5D^{CRP} provided both the CBC and the CRP results from just 20 μ l of whole-blood samples in 1.5 minutes without the need for additional time for serum processing [11]. This short time of examination allows physicians to obtain the results within 15 minutes after sampling so that clinicians can make quicker clinical decisions (Figure 4).

Conventional time-consuming workflow



Fast results for an immediate treatment decision



FIGURE 4 Workflow of CRP testing from whole-blood and serum samples [11]

The Abbott Alinity CI Series, a clinical chemistry analyzer utilizing serum samples, is regularly applied for CRP testing at Dr. Soetomo General Hospital, Surabaya. The CRP reagent used at Dr. Soetomo Hospital is the Wide Range CRP 48 Vario Reagent Kit with a measurement range of 0.1 mg/dL-48 mg/dL, while the Humacount 5D^{CRP} has a range of 0.02 mg/dL-32 mg/dL [8]. The Abbott Alinity CI series and Humacount 5D^{CRP} both employ the same principle of immunoturbidimetry. In this method, as shown in Figure 5, anti-human CRP antibodies that are attached to latex particles bind to CRP antigens present in the sample, resulting in the formation of antigen-antibody complexes. These complexes are then measured turbidimetrically at a specific wavelength, where the turbidity level is proportional to the CRP concentration in the sample [10,11]. As the Humacount 5D^{CRP}

utilizes the whole-blood samples, it requires an extra reagent to lyse erythrocytes before adding the test reagent containing anti-human CRP antibodies [11].

The whole-blood CRP values measured by the Humacount 5D^{CRP} are consistent with the serum CRP values obtained from the Abbott Alinity CI series, regardless of whether the hematocrit (HCT) levels are normal or abnormal. This consistency is achieved through the automatic hematocrit (HCT) correction technology integrated into the Humacount 5D^{CRP}. These results align with previous studies comparing whole-blood CRP and serum CRP measurements [9,11,13]. A study on the Mindray BC-7500 CRP, a hematology analyzer with CRP testing capabilities, found a strong correlation between CRP values measured by Mindray BC-7500 CRP and the IMAGE 800, a clinical

chemistry analyzer that uses serum samples ($r = 0.9962$). The Mindray BC-7500 CRP is equipped with Blood Cell Volume (BCV) correction technology, which adjusts for the volume interference caused by white blood cells, red blood cells, and platelets, ensuring that CRP measurements remain consistent with serum CRP values [5]. Similarly, the

Pentra MS CRP, another hematology analyzer with CRP testing, measures CRP levels from pre-lysed whole-blood samples and adjusts values to plasma concentration using hematocrit (HCT) correction. The Pentra MS CRP showed a strong correlation ($r = 0.9964$) with serum CRP measurements from the Hitachi LABOSPECT analyzer [13].

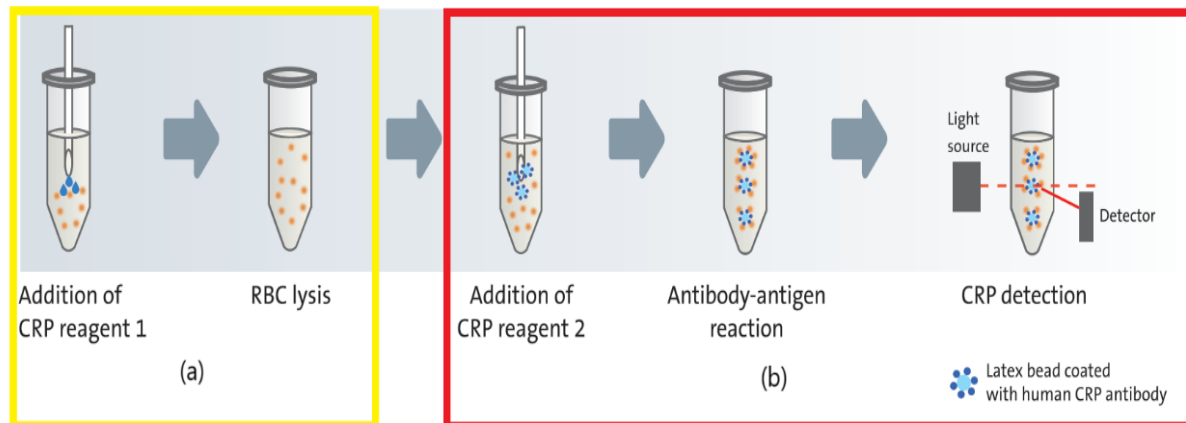


FIGURE 5 CRP testing methods on Humacount 5DCRP and Abbott Alinity CI Series; (a) additional steps for CRP testing with a whole-blood sample on Humacount 5DCRP and (b) CRP testing steps using the conventional immunoturbidimetry method with a serum sample on Abbott Alinity CI Series

Spearman test revealed a strong positive correlation ($R=0.956$, $p<0.001$), while Kappa agreement test analysis also demonstrated nearly perfect agreement between CRP levels on the Humacount 5DCRP and Abbott Alinity CI series ($R= 0.938$, $p<0.001$). These results indicate that whole blood CRP from the Humacount 5DCRP can serve as a reliable alternative to conventional serum CRP testing, especially in emergency settings, intensive care units, and outpatient clinics. It is particularly helpful in settings with moderate testing demands and less complex diagnostic requirements, where fast results remain essential. The Bland-Altman analysis that compared CRP measurements obtained from the Humacount 5DCRP and Abbott Alinity CI series found that among of 65 samples, only four samples had CRP differences exceeding the limit of agreement. These four samples showed higher CRP values on the Abbott Alinity CI series compared to the Humacount

5DCRP (34.05 vs. 17.41 mg/dL; 35.99 vs. 16.85mg/dL; 15.21 vs. 0.46 mg/dL; 42.25 vs. 14.92 mg/dL). Regardless of these variations, three out of the four samples remained in the high-CRP category.

This research did not specifically focus on cases with exceptionally elevated CRP levels. This limitation of the study is also highlighted out in similar research. A study comparing three CRP analysers revealed that the high levels of CRP produced different results. This difference was suspected to be assay-related, not population-based [14]. Another study comparing the Mindray BC-6800 and Johnson Vitros5600 analyzer also indicated a consistent deviation of CRP results between the two analyzers. The difference between the 2 analyzers may have been related to a certain difference in the performance of the different instruments [6]. The result discrepancy at high CRP levels can also be caused by differences in the measurement range of the

two analysers, so in future studies, dilution linearity can be carried out for extremely high CRP values [12]. Due to differences in assay at elevated levels, it is suggested to conduct further research in conditions with high CRP conditions, such as sepsis, trauma, and malignancy. The limited sample size in this study is another research drawback. Finally, future studies should focus on larger patient cohorts, extreme CRP values, and the effect of hematocrit variations to ensure the widespread clinical applicability of whole-blood CRP testing.

Conclusion

The study demonstrates a robust correlation between whole-blood CRP values measured using the Humacount 5D^{CRP} and serum CRP values measured using the immunoturbidimetry method (Abbott Alinity CI series, Abbott, Illinois, U.S.A). The Humacount 5D^{CRP} can serve as a reliable alternative to serum CRP testing. Aside from its analytical accuracy, the Humacount 5DCRP offers significant operational advantages, including reduced sample volume, shorter processing time, and decreased overall costs. These features make it particularly beneficial in clinical settings requiring rapid turnaround times to support timely decision-making and enhanced patient care. However, a significantly high result should be carefully interpreted.

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Authors' Contributions

All authors have made significant contributions to the study

Conflict of interest

The authors declared that there was no conflict of interest in this study.

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