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ORIGINAL ARTICLE

A Corrective Method for Different Hematocrit Values of Whole Blood Samples on the Detection of Procalcitonin

Yunyan Zhang [#, 1], Huiyan Zhuo [#, 2], Liu Yang [3], Jingjing Wang [2], Zhou Rui [2], Shuang Huang [2], Yunduan Wang [2], Xueya Zhang [4], Saixu Huang [4], Youqiang Li [2]

[#] *Yunyan Zhang and Huiyan Zhuo contributed equally to this work*
[1] *Department of Stomatology, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, Guangzhou, P.R. China*
[2] *Department of Laboratory Medicine, He Xian Memorial Hospital, Southern Medical University, Guangzhou, Guangdong, P.R. China*
[3] *Guangzhou University of Chinese Medicine, Guangzhou, P.R. China*
[4] *Research and Development Department, Shanghai i-Reader Biotech Co., Ltd, Shanghai 201114, P.R. China*

SUMMARY

Background: **We have explored that quantitative PCT detection can be conducted in different sample types (whole blood and/or plasma samples) with good correlation and consistency in clinical use. These findings reduce the sample volume and turnover time of PCT detection in clinical labs. However, different hematocrit (HCT) percentages of whole blood samples may affect the final results, especially abnormal hematocrit (HCT) percentages. To overcome this problem, we established a mathematical model to modify the whole blood test results and evaluated the effects of HCT correction.**

Methods: **First, we prepared a preliminary experiment - various hematocrit (HCT) percentages (15% - 65%) of whole blood samples with different PCT concentrations and established a mathematic model to correct the effects of PCT detection. Then, in this paper, we evaluated the consistency with Pearson's correlation and Kappa analysis between whole bloods detected by the i-Reader S system and plasma detected by the Biomerieux system. Besides, we prepared different HCT values about 15%, 40%, 60% of 9 samples with different PCT concentrations to evaluate the effects of HCT correction**

Results and Conclusions: **Pearson's correlative studies and Kappa analysis indicated that PCT levels measured by i-Reader S (plasma & whole blood samples) were comparable to results from the VIDAS system, and HCT correction could improve consistency of PCT detection between whole blood and plasma. Analysis of samples with abnormal HCT values showed that the mathematical correction model could offset the influences of various HCT values.**

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Correspondence:
Youqiang Li
Department of Laboratory Medicine
He Xian Memorial Hospital
Southern Medical University
Guangzhou, Guangdong, P.R. China
Email: liyouqiang21@126.com

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INTRODUCTION

Procalcitonin (PCT), a precursor of calcitonin, is a polypeptide containing 116 amino acids with a molecular weight of 13,000 Da. PCT is produced by C cells of the thyroid in healthy individuals [1,2]. As a biomarker, the level of PCT is able to reflect the presence and severity of local and systemic bacterial infection, commonly

used to monitor the effectiveness of antimicrobial therapy and the prognosis of infection [3,4]. Normally, the blood concentration of PCT is below 0.1 ng/mL in a healthy individual; however, PCT level increases to thousands of times higher than normal physiological conditions in response to pro-inflammatory stimulation [5,6], such as severe parasitic, bacterial or fungal infection. The cut-off value of 0.5 ng/mL is general accepted for adults and children while the PCT level > 0.5 ng/mL strongly suggests the presence of significant bacterial infection, sepsis likely [7].

Currently, serum and plasma samples are widely used for PCT detection. The application of these samples requires long preparation time for centrifugal separation and large volume of blood sample. To save sample preparation time, PCT detection in whole blood samples is becoming a more popular way in clinical use, even the peripheral blood sample can be detected directly. However, different hematocrit (HCT) values of whole blood samples may be one of the contributing factors that impair the accuracy of detection results, especially on the lateral flow assay platform. Lateral flow assay is almost the optimal method to detect proteins in whole blood samples due to the filtration of blood cells using sample pad, which removes the interferences of blood cells on quantification. Unfortunately, there are few studies currently available to address the problem of various hematocrit levels and PCT concentration cannot simply be calculated according to the HCT value and volume ratio due to the matrix effects caused by the dilution process. Therefore, a mathematical model including HCT value is of great importance to correct the measured PCT results in whole blood samples.

In this study, homologous whole blood and plasma samples were collected, and the HCT value of each sample was also recorded. Plasma samples were detected using i-Reader S immuno-analyzer and Biomerieux VIDAS system simultaneously, while whole blood samples were detected only using i-Reader S. According to the data fitting between the HCT percentage of whole blood and the PCT test results acquired by i-Reader S, a correction coefficient for whole blood samples with different HCT percentage was calculated. The mathematical model between correction coefficient and HCT value was proposed with data fitting. To verify the reliability of the model, Pearson's correlation and Kappa analysis were used to compare the PCT test results between different sample types (plasma and whole blood) and different systems (i-Reader S and Biomerieux VIDAS system). Samples with abnormal HCT values were also analyzed to verify the correction model. The results of statistical analysis indicated that the lateral flow assay-based PCT detection in plasma and whole blood samples could be realized using i-Reader S system with high consistency; more importantly, the accuracy of the detection results of whole blood samples can be improved using the HCT correction method. This study provides a novel approach for quantitative PCT detection in whole blood samples by combining lateral flow assays and HCT mathematical correction model, which will be helpful for achieving PCT detection in various sample types (serum, plasma, whole blood, and peripheral blood) with short turnaround time and less manual operation.

MATERIALS AND METHODS

Study design

Ninety-six samples with PCT levels ranging from 0.05 ng/mL to 40 ng/mL were collected in this study. Plasma samples were obtained from the clinical laboratory of the Hexian Memorial Affiliated Hospital of Southern Medical University. Patient data were anonymously used subject to the Helsinki Declaration of Human Ethics. Only stored samples were used in the retrospective study.

Collection of blood specimens

The collection of blood samples was carried out by professional phlebotomists in accordance with the International Standards of the Clinical Laboratory Standards Institute (CLSI) protocol GP41-A7 [8]. Whole blood samples were collected in EDTA-K2 anticoagulant tubes and plasma samples were obtained by centrifuging the blood at 4,000 r/min for 10 minutes. Samples with significant hemolysis, blood lipids, and jaundice were eliminated to minimize the interferences on test results.

In this study, samples were collected from 100 volunteers with suspected bacterial infection in He Xian Memorial Affiliated Hospital of Southern Medical University from 2020/07/26 to 2020/10/20. After removing the samples outside the detection range, 96 volunteers were included into the final statistical analysis.

Instrument and reagent

Tests were performed on the i-Reader S automatic immunoassay analyzer (Shanghai i-Reader Biotechnology Co., Ltd., Shanghai, China) and BioMérieux VIDAS system (BioMérieux, Marcy l'Etoile, France). Appropriate proprietary reference standard materials were used to calibrate the instruments. The PCT detection kits (PCT20003) and supporting sample dilution buffer were provided by Shanghai i-Reader Biotechnology Co., Ltd. (Shanghai, China). The multi-center evaluation shows that the coefficient of variation (CV) is less than 15% and the linear range is from 0.2 ng/mL to 30 ng/mL, and reportable range is from 0.2 ng/mL to 150 ng/mL. The detection time for each test is within 15 minutes.

Detection of plasma and 40% HCT whole blood samples

Plasma and red blood cells obtained by centrifugation were used to prepare the whole blood samples at the HCT percentage of 40%. The percentage of 40% is selected because the HCT level of healthy people is stable in normal condition, within a range of 40 - 45% [9]. The

PCT concentrations in plasma and prepared 40% HCT whole blood samples were determined by i-Reader S. By observing the detection results of 40% HCT whole blood and plasma samples, we calculated the theoretical ratio *a* as follows:

$a = C / C_{40\%}$

where *C* is the concentration of PCT in the plasma, $C_{40\%}$ is the concentration of PCT in the 40% HCT whole blood.

Determination of correction ratio for whole blood with different hematocrit percentages

Forty venous blood samples were collected in EDTA-K_2 anticoagulant tubes and centrifuged at 4,000 r/min for 10 minutes. Samples with significant hemolysis, blood lipids, and jaundice were eliminated to minimize the interferences on test results. Then the plasma and red blood cells obtained by centrifugation were used to prepare the whole blood samples at different HCT percentages from 20% to 65% with a 5% gradient (Each sample was not necessarily formulated into all HCT percentages.). The PCT concentrations in whole blood samples with different HCT percentages were detected by i-Reader S.

The correction coefficient γ for whole blood samples with different HCT percentage was calculated as follows:

$\gamma = Xi' / Xi$

where Xi is the concentration of PCT measured in the whole blood samples with HCT percentage ranges from 20% to 65% except 40%. *Xi'* is the concentration of PCT in the whole blood with 40% HCT percentage. The relationship between the correction coefficient γ and the HCT value can be established by performing data fitting.

Correlation analysis of different sample types

One hundred venous blood samples were collected and the HCT value of each sample was also recorded. Samples with significant hemolysis, blood lipids, and jaundice were eliminated to minimize the interferences on test results, and then these samples were tested using i-Reader S. The PCT concentration result of each sample is recorded as Ci.

The corrected PCT concentration *C'* with different HCT percentage could be calculated as follows:

$C' = Ci*a*\gamma$

The preparation of samples with abnormal HCT values

Nine venous blood samples were collected in EDTA-K_2 anticoagulant tubes and centrifuged at 4,000 r/min for 10 minutes. Then the plasma and red blood cells obtained by centrifugation were isolated to prepare whole blood samples with HCT value at 15%, 40%, and 60%. These samples were determined using i-Reader S and the result of PCT concentration is recorded as Ci.

The theoretical PCT concentration *C* is calculated based on HCT value as follows:

$C = C_{40\%} * a = Ci*a*60\% / (1-HCT)$

The corrected PCT concentration *C'* could be calculated as follows:

$C' = Ci*a*\gamma$

Statistical analysis

HCT values and PCT concentrations were presented as the mean ± SEM. The experimental data were processed with SPSS 17.0 and Microsoft Excel 2007 ($p < 0.05$ was considered as statistically significant). Consistency analysis was carried out by Bland-Altman plotting and Kappa test.

RESULTS

Mathematical model regarding the relationship between hematocrit (HCT) value and correction coefficient

The goal of our study was to develop a novel approach for direct PCT quantification in whole blood samples. Considering the effects of different HCT values on whole blood results, a mathematical model containing correction coefficient γ and HCT value was raised to correct the PCT results in whole blood samples measured by i-Reader S. Correction coefficient γ refers to the ratio of PCT concentration at different HCT values to PCT concentration at 40% HCT, which represents the normal condition of samples from healthy people. According to the data fitting results, the mathematical model was proposed. As shown in Figure 1.

Pearson's correlation analysis of different sample types

In this study, plasma PCT detection was performed using both i-Reader S and Biomerieux VIDAS system, and whole blood PCT detection was performed on the platform of i-Reader S. The results determined by VIDAS system that traces to BRAHMS PCT LIA are used as the reference standard. Pearson's correlation analysis is used for processing data obtained from different blood sample types and different systems [10].

As shown in Figure 2, there is a good linear regression relationship between plasma PCT results measured by i-Reader S and VIDAS system, with a slope of 0.9460 (slope from 0.85 to 1.15 indicates good agreement), $R^2 = 0.9565$, $p < 0.05$.

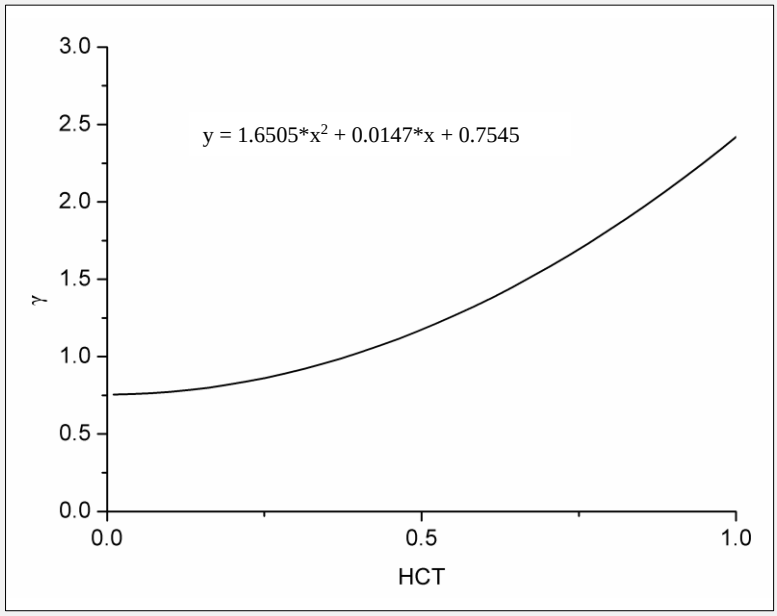
A similar correlation was observed between whole blood PCT results obtained from i-Reader S and plasma PCT results of VIDAS system, with a slope of 0.9203, $R^2 = 0.9512$, $p < 0.05$, as shown in Figure 3A. The slope data indicated that there is no significant deviation in PCT detection results between i-Reader S and VIDAS system, as well as the PCT detection results of whole blood and plasma samples measured by i-Reader S. Considering that different HCT values may affect PCT quantitative results, the results of whole blood samples should be adjusted. As mentioned above, the corrected PCT concentration can be described as $C' = Ci*a*\gamma$, according to the correction coefficient γ and ratio a. The

Table 1. Kappa analysis results of different sample types and different systems.

Blood samples	Negative or positive judgment	Biomerieux VIDAS system			Kappa	Positive proportion	
		positive	negative	total		plasma	81%
Plasma	positive	78	2	80	0.9286	plasma	83%
	negative	0	16	16			
	total	78	18	96			
Uncorrected whole blood	positive	77	4	81	0.8174	whole blood	84%
	negative	1	14	15			
	total	78	18	96			
Corrected whole blood	positive	77	1	78	0.9316	whole blood	81%
	negative	1	17	18			
	total	78	18	96			

Table 2. PCT results of whole blood samples with abnormal HCT value obtained by different measuring system.

Group	PCT concentration in VIDAS system	HCT	Correction	Uncorrected PCT	Corrected PCT
			coefficienty	Concentration in whole blood	Concentration in whole blood
1	1.22	11.90%	0.78	1.62	1.27
		35.50%	0.97	1.6	1.55
		57.40%	1.31	1.13	1.48
2	0.45	13.00%	0.78	0.51	0.4
		37.10%	0.99	0.4	0.4
		58.90%	1.34	0.32	0.43
3	3.47	13.20%	0.79	4.2	3.29
		33.40%	0.94	3.31	3.27
		58.40%	1.33	2.66	3.56
4	13.08	12.40%	0.78	16.19	12.66
		35.60%	0.97	11.67	11.3
		54.60%	1.25	8.49	10.65
5	3.09	13.70%	0.79	3.02	2.38
		37.10%	0.99	2.2	2.18
		57.20%	1.3	1.59	2.07
6	3.8	12.40%	0.78	3.54	2.77
		35.50%	0.97	2.67	2.58
		50.60%	1.18	2.21	2.62
7	0.25	14.40%	0.79	0.35	0.28
		36.20%	0.98	0.26	0.25
		55.00%	1.26	0.2	0.26
8	2.4	13.60%	0.79	3.27	2.58
		34.40%	0.95	2.75	2.63
		51.80%	1.2	2.3	2.77
9	1.16	13.70%	0.79	1.32	1.04
		40.10%	1.03	1.02	1.04
		49.50%	1.17	0.78	0.9



$$\gamma = 1.6505*HCT^2 + 0.0147*HCT + 0.7545$$

Figure 1. A mathematical model regarding the relationship between correction coefficient γ and HCT value.

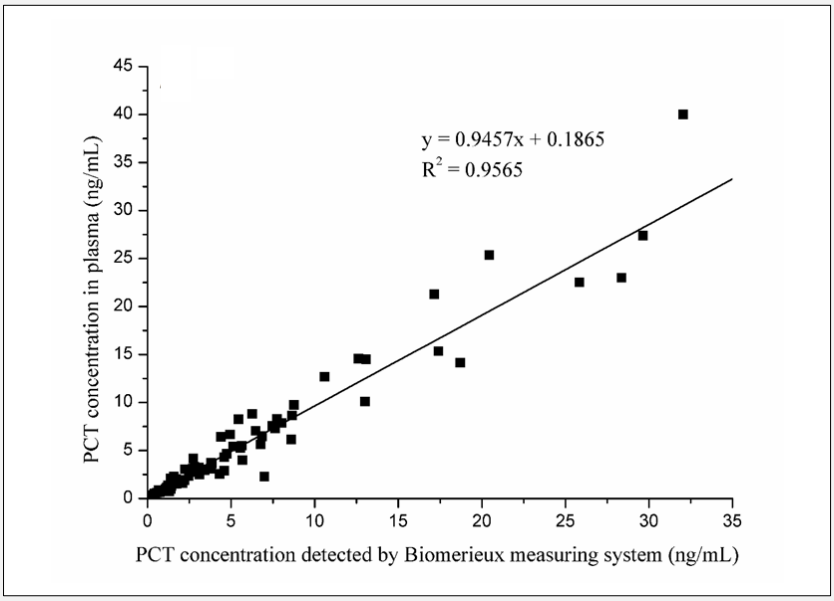


Figure 2. Pearson's correlation analysis of PCT concentration in plasma detected by i-Reader S system and Biomerieux system.

n = 96, concentration range 0.2 - 40 ng/mL, p = 0.05.

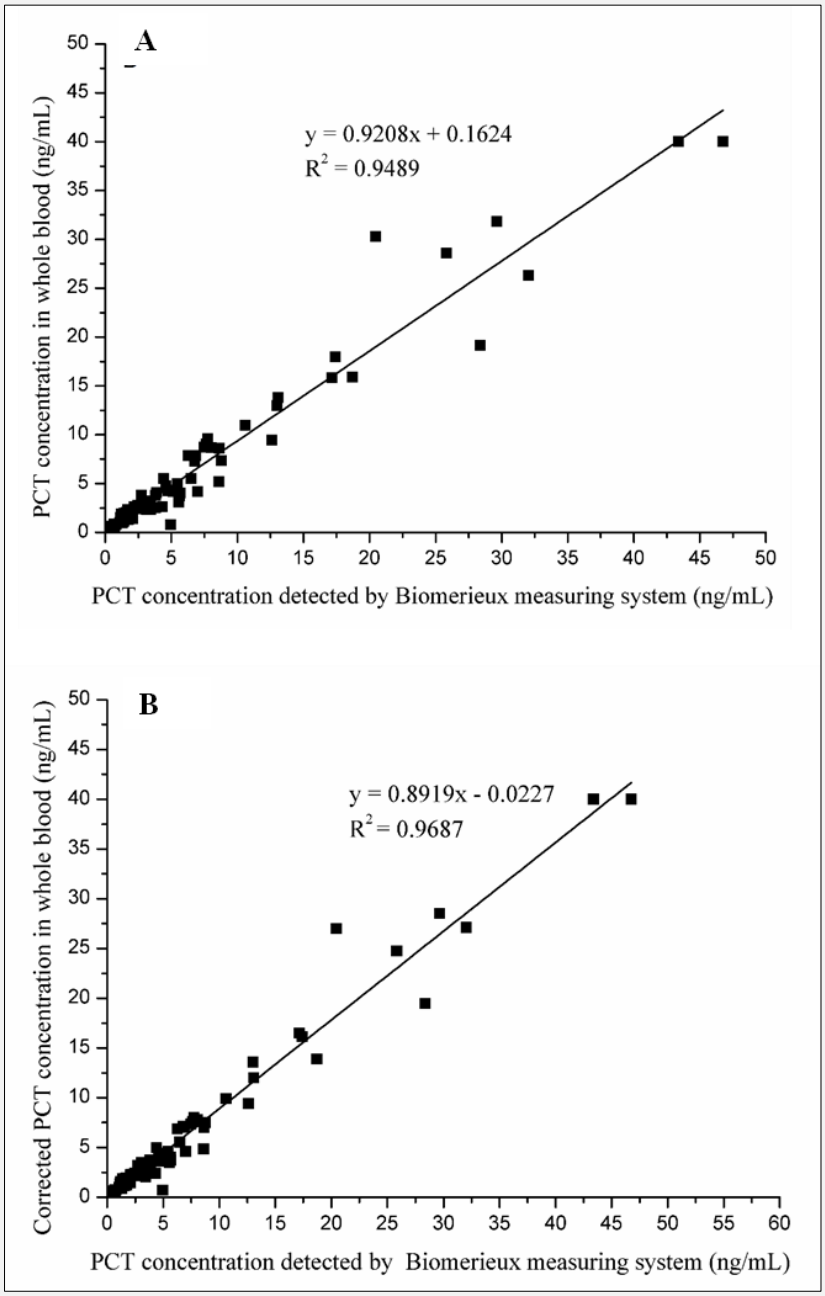


Figure 3. Pearson's correlation analysis of PCT concentration between whole blood detected by i-Reader S system and plasma detected by Biomerieux system.

Figure 3A, PCT concentration of whole blood detected by i-Reader S system without correction.
Figure 3B, PCT concentration of whole blood detected by i-Reader S with correction (n = 96; concentration range 0.2 - 40 ng/mL $p < 0.05$).

correlation analysis between corrected PCT concentration in whole blood samples and plasma results of VIDAS system was shown in Figure 3B, with a slope of 0.8914, $R^2 = 0.9706$, ($p < 0.05$). Compared with the results of uncorrected (Figure 3A) with a slope of 0.9208, $R^2 = 0.9489$, ($p < 0.05$), corrected PCT concentration had a better linear regression with VIDAS results. These results suggest that the mathematical model

for HCT correction is helpful for improving the accuracy of PCT detection results in whole blood samples. It is noteworthy that the process of PCT detection can be greatly improved with less sample volume and less preparation time by using the whole blood as sample type.

Kappa analysis of different sample types

In our studies, we used Kappa analysis to determine the consistency of PCT concentrations in plasma and whole blood samples detected by i-Reader S and VIDAS system. Using 0.5 ng/mL as cut-off value, the positive proportion of results detected by VIDAS system was 81%. The positive proportion of plasma and whole blood results detected by i-Reader S was 83% and 84%, respectively. The positive proportion of whole blood results after correction was 81%.

The kappa coefficient of plasma results detected by two different systems was 0.9286 ($p < 0.001$), indicating the almost perfect agreement of PCT detection between VIDAS and i-Reader S. Likewise, the kappa coefficient of whole blood results from i-Reader S and VIDAS plasma results was 0.8174 ($p < 0.001$), suggesting the substantial agreement of two different sample types, which is consistent with the observation of correlation analysis.

After the correction with correction coefficient γ and ratio a, kappa coefficient of whole blood results from i-Reader S and VIDAS plasma results was increased to 0.9316. Obviously, the mathematical correction model is able to improve the accuracy of PCT detection in whole blood samples.

In short, different sample types (plasma and whole blood) of PCT detection by i-Reader S had statistically significant linear correlation ($p < 0.05$) and good consistency with the results obtained by VIDAS system. HCT correction of i-Reader S system improved the accuracy of PCT detection in whole blood.

Analysis of samples with abnormal HCT values

To further confirm the applicability of the mathematical correction model, whole blood samples with abnormal HCT values were prepared. In this experiment, plasma samples were detected using VIDAS system while the homologous whole blood samples with abnormal HCT values were prepared and tested using i-Reader S. As shown in Table 2, uncorrected PCT concentrations were directly obtained by i-Reader S, corrected PCT concentrations were the results after calculation using the mathematical correction model and the theoretical PCT concentration was calculated according to the actual HCT value. Apparently, the corrected concentrations are closer to VIDAS system than the uncorrected results and theoretical PCT concentrations, especially when the HCT is around 15% and 60%. Also, the range of fluctuation caused by different HCTs is the smallest for corrected PCT results. The conclusion above demonstrated that the mathematical correction model greatly contributes to adjusting the whole blood results while the uncorrected results likely deviate from the true results under the conditions of abnormal HCT values.

DISCUSSION

The determination of PCT has found widespread application in the diagnosis and treatment of acute bacterial infection [11]. At present, serum and plasma samples are widely used for PCT detection in clinical use; however, time-consuming sample preparation and a large volume of blood sample are usually required. Therefore, we have developed a whole blood test method based on the lateral flow assay platform, which is the optimal approach for handling whole blood sample due to the filtration of blood cells on the sample pad. A whole blood-based test could significantly reduce the sample volume and turnover time of PCT detection in clinical laboratories.

However, different HCT percentages of whole blood samples may affect the final results since various plasma volumes are left on the lateral flow strip after the blood cell filtration with the constant sample adding volume. To overcome this problem, a mathematical correction model containing HCT values was proposed to modify the whole blood test results.

In this study, we collected whole blood and plasma samples from the same patient, and the HCT value of each sample was recorded. PCT concentration in plasma samples were detected by i-Reader S immuno-analyzer and Biomerieux VIDAS system, and in whole blood samples they were detected by i-Reader S.

In conclusion, different sample types (plasma and whole blood) for PCT detection by i-Reader S had statistically significant linear correlation ($p < 0.05$) and good consistency with the results detected by VIDAS system. HCT correction of i-Reader S system improved the accuracy of PCT detection in whole blood.

To further verify the effectiveness of the mathematical correction model at the conditions of abnormal HCT values, whole blood samples with abnormal HCT value at 15% and 60% were prepared. Compared to the uncorrected results and the theoretical PCT concentrations calculated based on HCT values, corrected results appear to be closer to the plasma results with better repeatability, which means that the mathematical correction model is able to provide more accurate whole blood PCT detection results with a wide range of HCT values.

In summary, PCT detection could be achieved using i-Reader S system in both plasma and whole blood samples based on lateral flow assay platform. One commonly raised problem about the whole blood test is the poor accuracy due to the variable HCT values from different samples, which may cause great deviation from homologous plasma results. Whole blood PCT detection is in rising demand since it allows reduced sample volume, less preparation time, and a simplified preparation process. To expand the application of whole blood samples, a mathematical correction model was proposed in

which the detection results are adjusted according to the actual HCT values.

We envision, PCT detection in whole blood will be developed in the following three aspects: 1) a small volume of sample requirement within the pediatric department; 2) short sample preparation time and clear clinical significance such as emergency department, ICU, and on the ambulance; 3) in combination with inflammatory biomarkers and routine blood test in the clinical laboratory, which would improve the sensitivity and specificity of bacterial infection diagnosis, further effectively guiding the use of antibiotics during disease treatment. Hopefully, the successful application of correction parameters on modifying the effects of HCT values will provide a universal strategy for designing whole blood-based detection of other critical biomarkers.

Ethical Approval:

Our research has "Human studies", for the necessity of human experiment, the protocol was approved by the ethics committee of He Xian Memorial Hospital, Southern Medical University, Guangzhou, Guangdong (2020 06001) and informed consent was signed by all patients or their close relatives. Before our study, we had inspected the feasibility of the experiment scheme from a large amount of literature. During the experiment, volunteers were ethically treated. The collection of blood samples was carried out by professional phlebotomists in accordance with the International Standards of the Clinical Laboratory Standards Institute (CLSI) protocol GP41-A7. Patient data were anonymously used subject to the Helsinki Declaration of Human Ethics.

Declaration of Interest:

The authors report no conflicts of interest in this work.

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