New Compact-Type Latex Photometric Immunoassay System for Hemoglobin and Three Acute Inflammation Markers: Neutrophil Count, C-Reactive Protein, and Anti-Streptolysin O

Tatsuo Fukumori,^{1*} Hironobu Ohta,² Akio Okubo,² Masayuki Hino,¹ Kensuke Ohta,¹ Takahisa Yamane,¹ and Noriyuki Tatsumi¹

¹Department of Clinical and Laboratory Medicine, Osaka City University Medical School, Osaka, Japan

²ARKRAY, Inc., Kyoto, Japan

A new compact-type latex photometric immunoassay system, SPOTCHEM IM SI-3510 (ARKRAY, Inc., Kyoto, Japan), which assays three kinds of inflammatory markers—neutrophil count (NPC), C-reactive protein (CRP), and anti-streptolysin O (ASO)—was evaluated. Hemoglobin (Hb), which is a good marker for anemia, can also be measured with it. NPC and CRP are measured using antibodies against neutrophilic elastase and CRP, purified streptolysin O was used for ASO determination, and Hb was measured by an azide-methemoglobin method. Whole blood, serum, and plasma specimens can be used as samples with this system. In this study, whole blood treated with dipotassium eth-

ylenediamine tetraacetic acid was used for evaluation. Linearity and reproducibility were good for all of the items studied. Good correlations were observed between the results obtained by this system and those obtained by routine methods. Since NPC exhibited a high correlation with the routine white blood cell (WBC) counts, it was judged to be useful as a substitute for WBC counting. Since this system is small and easy to operate, and evaluation revealed reliable results, it was judged to be practical for small laboratories, and satellite testing in hospitals and physicians' office laboratories for patients suspected to have acute inflammation. *J. Clin. Lab. Anal.* 16:95–102, 2002. © 2002 Wiley-Liss, Inc.

Key words: point of care testing (POCT); white cell count; laboratory test device; febrile patients; physician's office laboratory; SPOTCHEM IM; whole blood

INTRODUCTION

For treatment of febrile patients, rapid and accurate diagnosis is required. White blood cell (WBC) count and C-reactive protein (CRP) have been considered to be the best markers of acute inflammation (1). In some pediatric cases, streptococcus infection should be considered in a differential diagnosis, since the infection may result in cardiac or renal complications, and titration of anti-streptolysin O (ASO) is also essential for diagnosing rheumatic fever (2). Therefore, these three inflammation markers (WBC, CRP, and ASO) are often used for febrile patients in the physician's office laboratory. WBCs can be counted very easily using hematology analyzers, but such instruments are relatively large in size and expensive. The problem with determination of CRP and ASO is that it generally requires over 1 hr of test time with a chemistry analyzer. Adoption of point of care testing (POCT) systems is a recent trend in emergency hospitals and medical facilities to save turnaround time and laboratory space (3,4). Various types of chemical-strip tests based on the dry-chemistry principle are commonly used for POCT of urine, feces,

and blood chemistry. However, there are some problems with the use of dry chemistry, such as inevitable matrix effects (4,5). In this context, a compact instrument, SPOTCHEM IM, and the exclusive reagent SPOTCHEM i-Pack (ARKRAY, Inc., Kyoto, Japan), based on a new wet chemistry, was developed. Its performance was evaluated in this study.

MATERIALS AND METHODS

SPOTCHEM IM and SPOTCHEM i-Pack

The compact immunoassay system SPOTCHEM IM was developed for use in small laboratories as a POCT instrument (Fig. 1). This is a simple system that uses a reagent

*Correspondence to: Tatsuo Fukumori, Department of Clinical and Laboratory Medicine, Osaka City University Medical School, 1-4-3 Asahimachi, Abeno, Osaka, 545-8585 Japan. E-mail: noritatsumi@med.osaka-cu.ac.jp

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Fig. 1. Flow diagram of the latex photometric immunoassay system.

pack which combines all the items required for measurement, such as reagents, a distribution tip, and a disposal tank. An exclusive reagent pack is available for each measured item. The apparatus is 330 mm wide, 224 mm deep, and 185 mm high; it weighs 6 kg. It has three wavelengths and a light emission diode (LED) source (568, 660, and 850 nm), and is capable of processing six tests per hour. It is designed to perform calibration for different reagent packs by insertion of a magnetic card to store the lot information for each reagent pack.

Principle of the Procedure

Neutrophil count (NPC)

Whole blood is first lysed by saponin in the reagent. Neutrophilic intracellular elastase in the lysate reacts with latex particles coated with mouse monoclonal antibody to human neutrophilic elastase (buffer condition: pH 9.0; mixing ratio of whole blood vs. reagent: 1:3,600; reaction time: 5 min; temperature: 37°C). Particles aggregated by the reaction are measured turbidometrically to calculate the elastase concentration in the blood. The elastase concentration in whole blood showed a good correlation to NPC with routine methods (Fig. 2) (6). We made use of this correlation to convert elastase concentration to NPC. The elastase concentration is first converted to the NPC. Then the NPC is converted to conventional WBC count using a correlation factor between the WBC count and the NPC.

CRP

Similarly, CRP in plasma is measured turbidometrically using latex particles coated with goat polyclonal antibody to human CRP (buffer condition: pH 8.4; mixing ratio of whole blood vs. reagent: 1:300; reaction time: 5 min; temperature: 37°C).

ASO

ASO is also measured turbidometrically using latex particles coated with purified streptolysin O (buffer condition:

Fig. 2. Correlation between NPC obtained by the routine method and neutrophilic elastase concentration with the tested method.

pH 8.1; mixing ratio of whole blood vs. reagent: 1:300; reaction time: 5 min; temperature: 37°C).

Hemoglobin (Hb)

Hb is determined with an azide-methemoglobin method at a wavelength of 568 nm (7) (mixing ratio of whole blood vs. reagent: 1:130; reaction time: 2 min; temperature: 37°C). The hematocrit is obtained from Hb, and is used to calculate plasma volume (8) so that the CRP and ASO values for whole blood can be converted to those in the plasma.

Determination Procedures

The reagent pack, which has a reaction chamber, is set on the apparatus. One drop (100 μ L) of whole blood is put into a sample hole in the reagent pack (which is made of polystyrene), and then the start button is pressed for measurement. An aliquot of the blood (10 μ L) automatically enters the reaction chamber, attenuated without manual operation. All reactions are processed in liquid-phase in the reaction chamber. Turbidity induced by particle aggregation is measured with an LED at a wavelength of 660 nm, and Hb coloring is measured at 568 nm. The results are printed out 13 min after sample loading, by a built-in thermal printer.

Specimens

Nonleukemic specimens were obtained from outpatients visiting or admitted to the Department of General Medicine, while leukemia specimens were obtained from the Department of Clinical Hematology of Osaka City University Hospital. The specimens were collected using a vacuum tube containing dipotassium ethylenediamine tetraacetic acid (Terumo Corp., Tokyo, Japan).

The specimens were collected for routine hematology tests and these were treated according to the ethics code of the

Fig. 3. Linearity tests for NPC, CRP, ASO, and Hb.

department. All evaluation tests were completed within 6 hr after blood collection unless otherwise stated.

Linearity, Reproducibility, and Stability of Specimens, and Sensitivity and Interference Tests

Linearity tests were carried out by diluting specimens in five steps with autologous plasma. Reproducibility was evaluated by 10 repeated tests for each specimen, and the coefficient of variation was calculated. The stability of specimens in storage was tested at room temperature and at 4°C at 0, 1, 8, and 24 hr after blood collection. Sensitivity of this assay is defined as the smallest single value that can be distinguished from zero standard with a 99.5% confidence level.

TABLE 1. Reproducibility in ten consecutive tests

	Specimen 1	Specimen 2	Specimen 3
WBC			
Mean ($\times 10^3/\mu\text{L}$)	4.12	8.22	15.65
SD	0.123	0.132	0.420
CV (%)	2.98	1.60	2.68
CRP			
Mean (mg/dL)	1.10	3.20	10.79
SD	0.03	0.00	0.07
CV (%)	2.90	0.00	0.68
ASO			
Mean (IU/mL)	109	282	524
SD	1	5	9
CV (%)	0.78	1.67	1.66
Hb			
Mean (g/dL)	5.26	14.90	19.56
SD	0.18	0.17	0.11
CV (%)	3.38	1.14	0.55

SD, standard deviation; CV, coefficient of variation; WBC, white blood cell count; CRP, C-reactive protein; ASO, anti-streptolysin O; Hb, hemoglobin.

Interference tests for CRP, ASO, and Hb determinations were conducted using specific reagents of plasma component concentrates (Kansho Check, International Reagents Co., Kobe, Japan). Reagents containing high concentrations of unconjugated bilirubin (0, 5, and 20 mg/dL), conjugated bilirubin (0, 5, and 20 mg/dL), triglyceride (0, 200, and 600

Fig. 4. Stability tests with storage at room temperature and 4°C.

Fig. 5. Interference tests for CRP.

mg/dL), ascorbic acid (0, 10, and 50 mg/mL), and rheumatoid factor (0, 50, and 100 IU/mL) were used. Interference tests were conducted using specimens with high or low con-

Fig. 7. Interference tests for Hb.

centrations of CRP, ASO, and Hb. For NPC, interference was observed using specimens with hyperglycemia (339–726 mg/dL) or hyperbilirubinemia (11.5–23.3 mg/dL) obtained from the patient group.

Comparability to Routine Results

The data obtained with the SPOTCHEM IM were compared with those obtained by routine laboratory measurements in the Department of General Medicine of Osaka City University Hospital. WBC, Hb, and WBC differentials were obtained using an SE-8000 automated hematology analyzer (Sysmex Corp., Kobe, Japan). Specimens with abnormal flags were analyzed in the traditional microscopic fashion using Giemsa-stained films to obtain WBC differentials. The reference NPC obtained by the routine method was calculated as $[(\text{WBC obtained by the routine method}) \times (\text{percentage of neutrophils obtained by the routine method})]$. The NPC for the tested method was obtained by the instrument in the process of obtaining the WBC count. The CRP was measured using an LPIA-Ace CRP-L latex photometric immunoassay kit (Dia-Iatron Co., Ltd., Tokyo, Japan) and a HITACHI-7450 automatic multipurpose analyzer (Hitachi, Ltd., Tokyo, Japan). The ASO was measured using an N-Latex ASL latex photometric immunoassay kit (Dade Behring, Marburg, Germany) and an LX-3000 latex agglutination instrument (A&T Co., Tama, Japan).

The SPOTCHEM IM was evaluated in an office laboratory in a general hospital. Values of WBC, Hb, and CRP for

Fig. 6. Interference tests for ASO.

Fig. 8. Correlation between data obtained by the routine method and those with the tested method for NPC.

patients with fever over 37.5°C were collected using the routine method and the SPOTCHEM IM. In the general hospital, CRP was measured in a commercial lab in which a HITACHI-7150 (Hitachi, Ltd.) and the reagent LZ Test Eiken CRP (Eiken Chemical Co., Ltd., Tokyo, Japan) were used. WBC and Hb were determined using the small hematology analyzer K-1000 (Sysmex Corp). A comparison study for ASO was not performed, because only a few specimens were assayed during the study period.

RESULTS

Linearity, Reproducibility, and Stability of Specimens, and Sensitivity and Interference Tests

Linearity tests (dilution tests) demonstrated excellent linearity for all items, as shown in Fig. 3. Reproducibility tests

Fig. 9. Correlations between data obtained by the routine method and those with the tested method for WBC.

were conducted for three specimens, and the results revealed excellent reproducibility with low coefficients of variation (Table 1). The stability of the specimens in storage was tested, and the values obtained were stable during storage up to 24 hr, although NPC decreased by 24 hr of storage at room temperature (Fig. 4). Sensitivity was 0.2 mg/dL for CRP, 20 IU/mL for ASO, 600 cells/μL for NPC, and 1 g/dL for Hb. When unconjugated bilirubin, conjugated bilirubin, triglyceride, ascorbic acid, or rheumatoid factor was observed in CRP, ASO, and Hb determinations. All the results fell into 10% range of influence rate except for a specimen of low CRP which is obtained by the addition of ascorbic acid (Fig. 5–7).

Comparison

A high correlation was observed between the reference NPC and the test NPC values obtained with our system, as shown in Fig. 8. In general, WBC count, rather than NPC, is used in clinical practice. Thus, NPC was converted with our computer program to an estimated value of the WBC count, and the estimated WBC count was compared to the reference WBC count.

Interestingly, the WBC values estimated by our system exhibited almost the same *r* value as the correlation curve shown in Fig. 8 for the specimens obtained from outpatients (Fig. 9). Also specimens with hyperglycemia (>300 mg/dL, *n* = 13) or hyperbilirubinemia (>10 mg/dL, *n* = 5) were also measured. All the results showed good correlations with routine method (Figs. 8 and 9). However, since neutrophilic elastase is not contained in immature leukocytes but is contained in neutrophils (9), it can be assumed that leukemia specimens exhibit incorrect WBC counts. To confirm this, lymphoid and myeloid leukemia specimens were selected and tested. As Table 2 shows, leukemia cases had low WBC counts. These results suggested that leukemia specimens were not suitable for our testing system.

Figure 10 shows comparisons for CRP, ASO, and Hb. For all parameters, good correlations were observed between the data obtained by SPOTCHEM IM and those by the routine method.

Since the present system is designed for clinics and small laboratories, its utility was evaluated in a site involving treatment of febrile outpatients. Figure 11 shows the clinical course of changes in WBC and CRP in a case of febrile infection.

TABLE 2. Comparison of white blood cell counts (WBC) obtained by the routine method and turbidoimmunometry in leukemia specimens

Type	n	Routine method				Tested method	
		blast %		WBC		WBC	
		Mean (%)	SD (%)	Mean (×10 ³ /μL)	SD (×10 ³ /μL)	Mean (×10 ³ /μL)	SD (×10 ³ /μL)
L	9	15.6	17.8	6.5	3.63	2.0	1.05
M	15	40.3	28.60	20.5	21.56	2.83	1.86

SD, standard deviation; L, lymphocytic leukemia; M, myeloid leukemia.

Fig. 10. Correlations between the routine data and those obtained by the tested method for CRP, ASO, and Hb.

WBC and CRP values shifted in parallel with both the routine method and the tested method. In our practical trial, good accordance was obtained between reference methods and our tested methods for WBC, CRP, and Hb determinations (Fig. 12). In the observation, all data distributed well along the regression lines regardless of disease-type differences.

DISCUSSION

Infection or tissue injury induces an inflammatory response that is accompanied by a systemic response, the so-called systemic inflammatory response syndrome (10), which includes induction of fever, increased production of WBCs, and production of a large number of hepatocyte-derived acute-phase proteins, including CRP and serum amyloid A (11). Physicians working in emergency departments or polyclinics in the Department of General Medicine are required to determine the causes of fever when they see febrile patients, and WBC and CRP are clearly good indicators for screening of acute infection. If WBC and CRP tests could be performed simply and rapidly, it would be very useful for such physicians. In this context, the SPOTCHEM IM was developed and evaluated in this study. The ability of SPOTCHEM IM to determine WBC counts and CRP was judged to be reasonably good.

Current hematology analyzers use an impedance or optical particle counting system to provide very accurate and precise data (12). Thus, it might not be suitable to determine WBC with immunoassay in some aspect. However, such a turbidometric method has commonly been used for various types of qualitative immunological testing, and is widely used in the determination of hormones. This technology was transferred to the present cell-counting method, and yielded acceptable results for WBCs. Saponin hemolysis, which is used in the present instrument, is an established method for counting WBCs (13). Neutrophilic elastase (EC 3.4.21.37) is a cationic glycoprotein in azurophilic granules of human neutrophils (content: 1–4 $\mu\text{g}/10^6$ cells), and has biochemical

Fig. 11. Clinical course of changes in WBC and CRP in a febrile patient.

Fig. 12. Correlation between the routine data and those obtained by the tested method for febrile patients.

characteristics different from those of pancreatic elastase (EC 3.4.21.36) in various respects, such as molecular weight and structure. It has strong bactericidal activity and enhances activities of neutrophilic enzymes such as lysozyme and cathpsin G (14,15). The SPOTCHEM IM measures total enzyme content in WBC lysate using anti-elastase antibody. The concentration obtained is converted first to NPC, which is used to estimate the WBC count. The estimated WBC count is an acceptable estimate of the number of WBCs, but would be most suitable when used for limited purposes, such as in the emergency department or small laboratory of the practitioner. Lymphocytic cells and blastic cells have no esterase-containing azunophilic granules (15). Leukemia is an uncommon disease and can be diagnosed with parameters other than WBC count, such as erythrocytopenia, thrombocytopenia, and other clinical signs. Therefore, an estimated WBC count is not suitable for detecting lymphocytosis or leukemia. However, many hematology analyzers with three-part differential functions are also unable to determine the existence of leukemia, and can only exhibit suspect flags for abnormalities (12). Since the SPOTCHEM IM is targeted for rapid diagnosis of acute inflammation and neutrophilia, the estimated WBC count would primarily be useful for outpatient or emergency clinics.

CRP, a representative acute phase reactant, has been measured in the clinical laboratory immunonephelometrically or immunoturbidometrically (16). In addition to its use for acute inflammation (17,18), CRP is now used for diagnosing connective tissue diseases such as rheumatoid arthritis, inflammatory bowel disease, neonatal infection, myocardial ischemia, coro-

nary artery disease, and tumors (19–22). The currently used methods for CRP determination are highly reproducible and fully automated, with detection limits less than 30–50 mg/dL for CRP. Hence, CRP is a nonspecific inflammation marker, although CRP is increased in association with changes in WBC.

Hb determination is included in the SPOTCHEM IM for clinical convenience in determining anemia and correcting CRP and ASO values in plasma. The Hb detection principle used involves a nonbiohazardous azide-methemoglobin method, and the results obtained indicated excellent performance.

Our data, obtained from patients in a general hospital, demonstrate the clinical and diagnostic usefulness of SPOTCHEM IM at the practical level. Whole blood treated with ethylenediamine tetraacetic acid was used for the present method. Also, heparinized plasma was also used for the present method of CRP and ASO, both results were good as expected (data obtained are not shown here). Troublesome operations, such as washing of flow lines are not required, because everything required for determination is included in the reagent pack, and the apparatus is very simple. Therefore, the SPOTCHEM IM can be operated easily, without an expert technician. With its high reproducibility and linearity, good correlation with routine methods, lack of interference, small size, and simple test procedures, the SPOTCHEM IM should be suitable for practical use in POCT.

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