Letter to the Editor
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Effects of Cold Agglutinin on the Accuracy of Complete Blood Count Results and Optimal Sample Pretreatment Protocols for Eliminating Such Effects

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Dear Editor,

Cold agglutinin is an autoantibody that causes autoimmune hemolytic anemia by binding to I/i carbohydrate antigens on the red blood cell (RBC) surface [1]. While RBC agglutination causes clinical symptoms of hemolytic anemia, agglutination caused by cold agglutinin is a notorious pre-analytical and analytical factor that leads to spurious automated complete blood count (CBC) results [2]. Although RBC agglutination falsely increases mean corpuscular volume (MCV), effects of cold agglutinin on other CBC parameters have not been widely studied [3, 4]. The effects of cold agglutinin on CBC results have been evaluated mainly for a limited number of parameters, on old versions of automated CBC analyzers [5, 6], which have undergone remarkable technical improvements in the last decade. We present a case of chronic cold agglutinin disease, and report the effects of cold agglutinin on CBC parameters based on whole blood samples comparatively analyzed by four automated CBC analyzers commonly used in current clinical laboratories.

A 56-year old male patient visited our hospital for low hemoglobin (Hb) concentration, fatigue, and hematuria. The initial CBC results revealed the following: Hb, 102 g/L; platelets, 275 $\times 10^9$/L; and white blood cell (WBC) count, 4.70$\times 10^9$/L. Peripheral blood smear showed normocytic normochromic anemia with mild anisopoikilocytosis, mild elliptocytosis, and dacryocytes. RBC clumps that resolved after sample incubation at 37°C suggested the diagnosis of cold agglutinin disease. The patient required no specific treatment because symptoms were not severe. Follow-up seasonal variations in symptoms and CBC results were observed. The institutional review board of National Health Insurance Service Ilsan Hospital approved this study (IRB number: NHIMC 2015-03-011).

In total, 16 K_2EDTA samples of whole blood were collected. The first sample was analyzed on the XE-2100 (Sysmex, Kobe, Japan) immediately after collection, the results of which served as reference values. The four most commonly used automated CBC analyzers—XE-2100, XN-1000 (Sysmex), ADVIA 2120i (Siemens Diagnostics, Tarrytown, NY), and Unicel DxH 800 (Beckman Coulter Inc., Fullerton, CA)—were used for comparative analysis. The experimental flowchart is shown in Fig. 1A.

Each automated CBC analyzer measured four samples. System accuracy was compared using the unit of delta percentage difference (DPD) from the reference value, which was defined

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according to a previous report [7] as follows:

$$\text{Delta Percentage Difference (\%)} = \frac{\text{(Test analyzer test tube – reference analyzer reference tube)}}{\text{Reference analyzer reference tube}} \times 100$$

We analyzed the effects of analyzer type, storage temperature, storage duration, and incubation period, using eight CBC parameters [RBC count, Hb, hematocrit, MCV, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), WBC count, and platelet count] in terms of DPD. We interpreted the results as positive or negative bias, or acceptable. The criteria for spurious effects were derived from three guidelines for quality requirements for automated CBC analyzers (Table 1) [8-10].

When we compared three temperature conditions [i.e., measurements (1) or (2), (3), and (4) in Fig. 1A, according to analyzer], the sample stored for 1 hour at 37°C showed the smallest deviance from the reference value based on the DPD value (Fig. 1B, Table 1). Interestingly, Hb concentration and platelet count were not highly affected by storage temperature, while WBC count showed variability according to the analyzer used. For all analyzers, except the XN-1000, sample storage at 37°C for 1 hour yielded more accurate results than storage at 37°C for 24 hours. When we compared samples stored at 37°C for 1 hour with samples stored at 4°C for 1 hour followed by incubation at 37°C for 1 hour, there was no significant difference in any of the parameter values (Fig. 1C). Based on these results, we propose two practically optimal protocols: (1) measuring CBC within 1 hour if the sample is stored at 37°C after collection and (2) measuring CBC after incubation at 37°C for 1 hour if the sample is stored at 4°C for a short term.

An interesting finding of our study was that Hb concentration showed the smallest variance among all parameters evaluated in this study. This finding is consistent with those of previous studies [2, 3] and is likely owing to the fact that we measured Hb after RBC lysis, which eliminates clumping by cold agglutinin. From a clinical aspect, it is reassuring that the most important variable for determining the need for RBC transfusion is not substantially distorted by the presence of cold agglutinin.

Thus, we demonstrated spurious effects of cold agglutinin on eight parameters comparatively measured on four automated CBC analyzers. Our results indicated that cold agglutinin affects not only MCV but also other parameters, depending on analyzer type. Optimal pretreatment protocols to eliminate spurious effects of cold agglutinin on results generated by automated CBC analyzers are needed.

Table 1. Cut-off values for interpretation of DPD values derived from three quality requirement guidelines and effects of storage temperature and duration on parameters based on DPD compared with acceptable criteria

Measurement time in Fig. 1A	Unit	A range for interpretation of equivalent result	Reference	XE-2100				XN-1000				ADVIA 2120i				Unicel DxH 800			
				(1)	(3)	(4)	(8)	(1)	(3)	(4)	(8)	(1)	(3)	(4)	(8)	(1)	(3)	(4)	(8)
RBC count	$\times 10^{12}$/L	Target value within 8.0%	5	N	N	A	A	N	N	N	A	N	N	A	A	N	N	N	N
Hb	g/L	Target value within 7.0%	9	N	A	A	A	N	A	A	A	A	A	A	A	A	A	A	A
Hematocrit	Proportion of 1.0	Target value within 9.0%	8	N	N	A	P	N	N	N	P	N	N	A	P	N	N	N	N
MCV	fL	Target value within 7.0%	10	A	A	A	P	A	A	A	P	A	A	A	P	P	P	P	P
MCH	pg	Target value within 5.0%	10	P	P	A	A	P	P	P	A	P	P	A	A	P*	P*	P*	P*
MCHC	g/L	Target value within 8.0%	10	P	P	A	N	P	P	P	N	P	P	A	N	P*	P*	P*	P
WBC count	$\times 10^{9}$/L	Target value within 18.0%	8	A	A	A	A	N	N	N	N	A	N	A	A	P	P	A	N
Platelet count	$\times 10^{9}$/L	Target value within 25.0%	9	P	A	A	A	A	A	A	A	P	A	A	A	P	A	A	A

*Values were calculated manually using other parameters because no report was available from the analyzer.
Abbreviations: DPD, delta percentage difference; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cell; WBC, white blood cell; P, positive bias; N, negative bias; A, acceptable.

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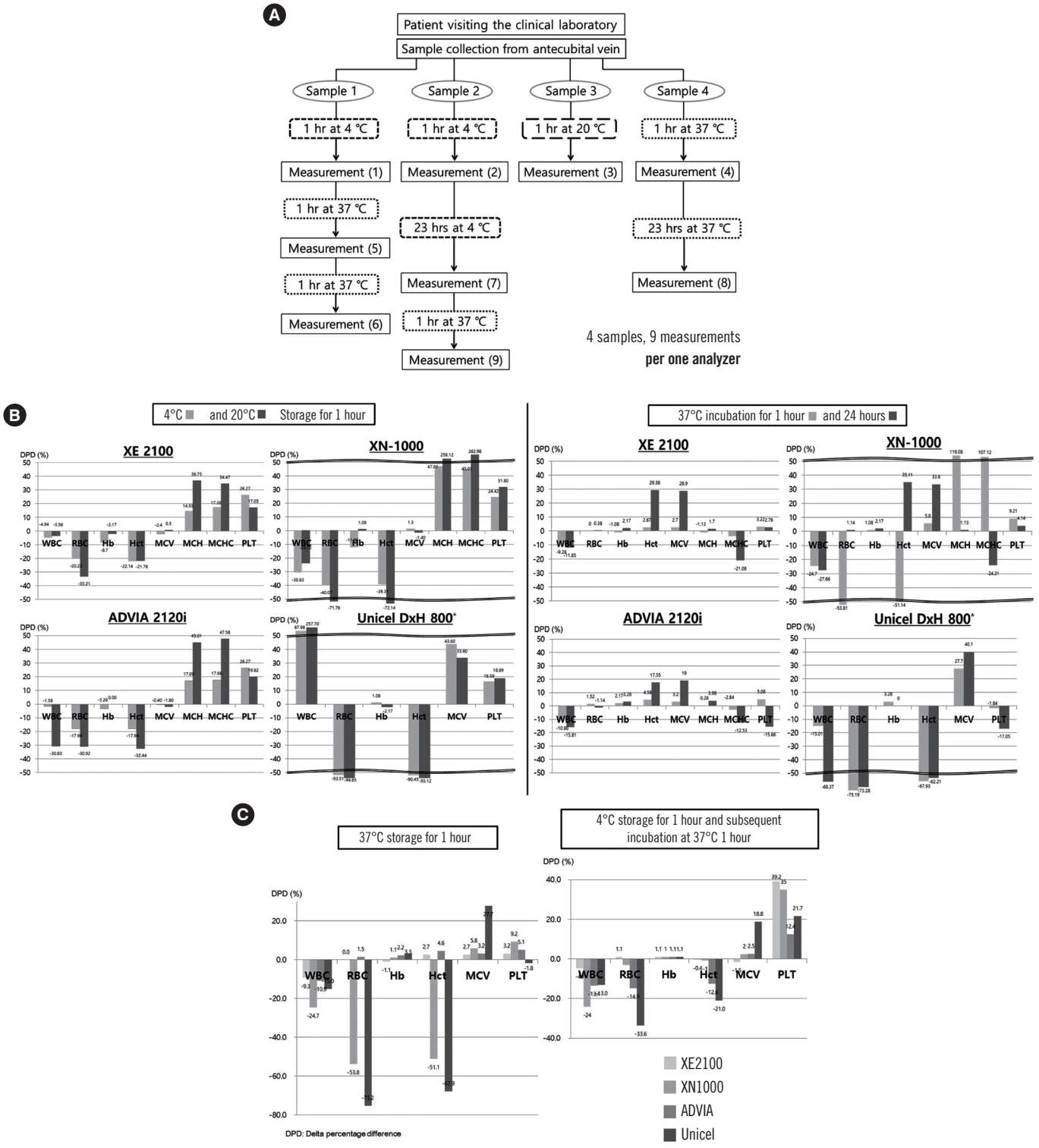

Fig. 1. Summary of study design and comparative assay results. (A) Experimental flowchart of sample collection and measurements. In total, four samples were evaluated in nine measurements per analyzer. Thus, 16 samples in total were evaluated 36 times, on four different automated CBC analyzers. (B) Effects of storage temperature and duration on CBC parameters measured with the four analyzers. (C) Comparison of the four automated CBC analyzers applying two optimal protocols, which were (1) measuring CBC within 1 hour of storage at 37°C and (2) measuring CBC after short-term storage at 4°C for 1 hour and subsequent incubation at 37°C for 1 hour.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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