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Screening and Identification of Unexpected Red Cell Antibodies by Simultaneous LISS/Coombs and NaCl/Enzyme Gel Methods

We evaluated the clinical usefulness of simultaneous LISS/Coombs and NaCl/Enzyme testing using the gel method for screening and identification of unexpected antibodies in 15,014 samples. When unexpected antibodies were detected by either screening test, those antibodies were identified using both the LISS/Coombs and the NaCl/Enzyme gel test. The positive screening rates of the LISS/Coombs, NaCl/Enzyme, and combined tests (excluding 25 autoantibody cases) were 0.48%, 1.29%, and 1.39%, respectively. Among the 57 samples positive by both screening methods, the antibodies in 19.3% could be identified only by the NaCl/Enzyme method. Among the 137 samples positive only by NaCl/Enzyme screening, 74.5% showed positive results in antibody identification only by the NaCl/Enzyme test, although 7.3% were also positive in the LISS/Coombs test. The NaCl/Enzyme method thus showed about threefold higher detection rates than the LISS/Coombs method, especially in screening for Rh antibodies, and higher exact identification rates and discriminatory power for identifying mixed antibodies. Addition of the NaCl/Enzyme method to routine laboratory procedures may detect and identify considerable numbers of significant antibodies that might be missed if only the LISS/Coombs method is used.

Key Words : *Erythrocyte Antigens; Unexpected Antibody; Gel Test; NaCl/Enzyme; Coombs' Test*

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INTRODUCTION

Clinically significant unexpected antibodies are capable of causing hemolytic transfusion reactions secondary to accelerated destruction of a significant proportion of transfused red blood cells (1). Therefore, screening for unexpected antibodies should be part of all pretransfusion testing, with antibody identification in the event of a positive result. In the 1990s, the microcolumn gel technique was introduced for screening and identification of such unexpected antibodies (2). This method is not only easy to perform and economical of time but also easy to standardize and read, so it has become the most common technique in the blood bank laboratories of many countries (3).

The two principal techniques for unexpected antibody screening and identification are the indirect antiglobulin and enzyme methods. The most frequently used method is the indirect antiglobulin with gel (LISS/Coombs), and the microcolumn assay technique using the LISS/Coombs gel test is the most popular for this purpose in Korea (4-6). In recent years, the enzyme gel method (NaCl/Enzyme) has been added for antibody identification in a few hospitals in Korea due to its higher and exact identification rate (7). However, the NaCl/Enzyme method is used only for antibody identification, so some unexpected antibodies could be missed in screening step. At present, there has been no study in Korea of antibody screening and identification using these two methods.

The purpose of the present study was to compare the results of the LISS/Coombs and NaCl/Enzyme methods for screening and identifying unexpected antibodies and to evaluate the clinical usefulness of simultaneous testing by these two methods.

MATERIALS AND METHODS

Performance of unexpected antibody detection

From May 2005 to April 2006, unexpected antibody screening was performed on 15,014 samples using the LISS/Coombs and NaCl/Enzyme gel tests. When unexpected antibodies were detected by either test, those antibodies were identified using both methods.

A 50 μL sample of 0.8% screening or identification cell reagent and 25 μL of patient serum were added to the microtube of each gel card. After 15 min' incubation at 37°C, the card was centrifuged for 10 min, and the reactions for agglutination were examined macroscopically on an illuminated

view box.

All tests were carried out using the DiaMed-ID Micro Typing System (DiaMed Ag, Cressier, Morat, Switzerland). For the LISS/Coombs screening method, the LISS/Coombs card and two test reagents ID-Diacell I-II (DiaMed Ag) were used. For the NaCl/Enzyme screening method, the NaCl/Enzyme card and three test reagents DiaCell I-II-III P (papainized) (DiaMed Ag, ID) were used.

When unexpected antibodies were detected by either test, those antibodies were identified using both methods. For the LISS/Coombs identification test, the LISS/Coombs card and ID-Panel test reagent (DiaMed Ag) were used. For the NaCl/Enzyme identification test, the NaCl/Enzyme card and the ID-Panel P test reagent (DiaMed Ag) were used.

Interpretation of results

An antibody screening result was defined as positive if one or both of the cell reagents agglutinated with the patient's serum in the LISS/Coombs test, and if one or more of the three cell reagents agglutinated with the patient's serum in the NaCl/Enzyme test. For antibody identification, we interpreted each method as positive if one or more of the 11 cell reagents agglutinated. The final identification was made as follows. When only one antibody was identified in the serum, we interpreted it as "identified" if all reactions in the 11 wells were consistent with the manufacturer's identification table and as "unidentified" if the reactions in some wells were discordant with the table. When two or more antibodies were present, we interpreted them as "identified" if all antibodies were identified exactly with each method, as "partially identified" if at least one antibody was identified exactly with each method, and as "unidentified" if none of them was identified exactly. If no agglutination reactions occurred in any of the 11 wells, we interpreted the result as "negative".

RESULTS

Unexpected antibodies were detected by at least one method in 234 of the 15,014 serum samples, including 25 autoantibody-containing samples (1.56%). The positive screening rates of the LISS/Coombs, NaCl/Enzyme, and combined tests for unexpected alloantibodies were 0.48% (n=72), 1.29% (n=194), and 1.39% (n=209), respectively (Table 1). The positive rate of the combined methods was about threefold that of the LISS/Coombs method only, and highly discrepant results were seen between these methods. Only 57 (27.3%) of the total 209 alloantibody cases were positive by both methods, whereas 137 (65.6%) and 15 (7.2%) were positive only by the NaCl/Enzyme and the LISS/Coombs method, respectively.

Among the 57 samples showing positive in both screening tests, 45 were also positive in both identification tests. On the other hand, 11 samples were positive only by the NaCl/Enzyme identification test, and these antibodies were anti-E (n=5), anti-Le^a (n=3), anti-C+e (n=1), anti-e (n=1), and anti-E+Le^a (n=1) (Table 2). One sample was negative in both identification tests.

Among the 15 samples having positive results only by the LISS/Coombs screening test, 7 samples were positive for the LISS/Coombs identification test only, 1 for the NaCl/Enzyme test only, and 2 for both tests. Among the 137 samples having positive results only by the NaCl/Enzyme screening method, 102 samples were positive for the NaCl/Enzyme identification test only. The 61 samples were finally identified as anti-C (n=3), anti-c (n=1), anti-C+K (n=1), anti-E (n=25), anti-E+c (n=2), anti-Le^a (n=27), and anti-Le^b (n=2). But other 41 samples were "unidentified" in the NaCl/Enzyme identification test. Ten samples showed positive results in the LISS/Coombs method as well as the NaCl/Enzyme method, and these were finally identified as anti-D (n=1), anti-E (n=1), anti-E+c (n=1), or anti-Le^a (n=4), with 3 being unidentified. Twenty-five samples were negative in both identification tests.

According to the final identifications, anti-Rhesus antibodies were the most common, and 78 antibodies were identified. These antibodies were exactly "identified" in 20 cases and "partially identified" in 12 cases by the LISS/Coombs method and were exactly "identified" in 72 cases and "partially identified" in 6 cases by the NaCl/Enzyme method (Table 3). Of the 40 anti-Lewis antibodies, all were identified by the

Table 1. Differences in unexpected antibody screening results* between LISS/Coombs and NaCl/Enzyme Gel Tests excluding 25 autoantibody cases

LISS/Coombs	NaCl/Enzyme		
	Positive	Negative	Total
Positive	57	15	72
Negative	137	0	137
Total	194	15	209

*Screening cells are composed of two cell reagents in LISS/Coombs and three cell reagents in NaCl/Enzyme test.

Table 2. Comparison of unexpected antibody screening and identification by LISS/Coombs and NaCl/Enzyme Gel Tests for 209 unexpected antibody screening-positive cases

Antibody screening	Antibody identification				
	Enzyme and Coombs positive	Enzyme only positive	Coombs only positive	Enzyme and Coombs negative	Total
Enzyme and Coombs positive	45	11	0	1	57
Enzyme only positive	10	102	0	25	137
Coombs only positive	2	1	7	5	15
Total	57	114	7	31	209

Table 3. Results of antibody identification by LISS/Coombs and NaCl/Enzyme Gel Tests

Antibody specificity	n	LISS/Coombs				NaCl/Enzyme			
		Neg	UnID	P-ID	ID	Neg	UnID	P-ID	ID
Rh system									
Anti-E	46	30	3		13				46
Anti-E+c	17	2	1	12*	2			5†	12
Anti-c	3	1	1		1				3
Anti-D	3				3				3
Anti-C	3	3							3
Anti-C+e	2	1			1			1‡	1
Anti-e	1	1							1
Lewis									
Anti-Le_a	34	30	2		2				34
Anti-Le_a+UnID	2		2					2§	
Anti-Le_b	2	2							2
Duffy									
Anti-Fy_a	1				1	1			
Anti-Fy_b+UnID	1				1		1		
Other									
Anti-C+Fy_a+Le_b	1			1‖				1¶	
Anti-E+Le_a	1	1							1
Anti-C+K	1	1							1
Anti-Xg_a	2				2	2			
Unidentified	89	73	16			33	56		
Total	209	145	25	13	26	36	57	9	107

*Anti-E+UnID (n=2) and Anti-E (n=10), †Anti-c, ‡Anti-e, §Anti-Le_a, ‖Anti-Fy_a, ¶Anti-C+Le_b.
Neg, negative; UnID, unidentified; P-ID, partially identified; ID, identified.

NaCl/Enzyme method. In the LISS/Coombs method, only 2 antibodies were identified. The anti-Fy_a, anti-Fy_b, and anti-Xg_a antibodies were identified only by the LISS/Coombs method.

DISCUSSION

Previously, screening for unexpected antibodies was performed with an indirect antiglobulin test or an enzyme test using a conventional tube method. However, the recently introduced gel test has proved to be more sensitive and has many advantages (8, 9). The use of LISS has increased the number of antibodies detected, and clinically important antibodies have been found in increasing numbers. In addition, the gel method is rapid, and interpretation of the results is easy. Therefore, the gel test gained widespread usage throughout the world, including Korea.

In this study, we compared the results of the LISS/Coombs and NaCl/Enzyme tests using the gel method for screening and identifying unexpected antibodies and evaluated the clinical usefulness of simultaneous testing by two methods. Of the 15,014 patient samples tested, 0.48% had a positive reaction with the LISS/Coombs screening method. This rate is similar to that in previous reports from Korea (4-6). The positive rate of antibody screening increased to 1.29% with the NaCl/Enzyme method and to 1.39% using these two tests together. This result indicates that the NaCl/Enzyme method is sensitive in detecting unexpected antibody, especially Rh antibodies.

Among the 137 samples showing positive results in NaCl/Enzyme screening only, 102 samples were also positive only with the NaCl/Enzyme identification method and 10 were positive with both the LISS/Coombs and the NaCl/Enzyme identification. Anti-Rh (n=35), anti-Le (n=33), and unidentified antibodies (n=44) accounted for NaCl/Enzyme screening-only antibodies. Twenty-five samples showed negative results in both the LISS/Coombs and NaCl/Enzyme identification methods. The decision about the clinical significance of NaCl/Enzyme screening-only positive results is difficult, and we should be careful in interpretation. In a few previous reports, the authors stated that the enzyme method revealed a high proportion of nonspecific reactions with uncertain clinical value, and "enzyme-only" antibodies lack clinical significance, so the method is not employed routinely by many laboratories (10, 11). In our study, the unidentified antibodies (44/137) and negative results (25/137) in identification also accounted for a high proportion among the NaCl/Enzyme screening-only positive results. These are considered nonspecific reactions, clinically insignificant, or both, although we could not evaluate individual patient data for clinical significance. However, we cannot completely accept the opinion that all NaCl/Enzyme screening-only positive results are insignificant. A few reports about antibody screening in preg-

nant women showed different results that enzyme-enhanced methods often detect low concentrations of anti-Rh antibodies not found by other methods (12, 13). In addition, there are some cases of acute hemolytic transfusion reaction (14) or delayed transfusion reactions (11, 15) caused by "enzyme-only" antibodies.

We found additional evidence supporting the importance of the enzyme method for antibody screening in this study. Among the 137 samples that were NaCl/Enzyme-only positive, 10 showed positive results with the LISS/Coombs identification method. We have confidence that these antibodies are clinically significant. These antibodies were identified as anti-D (n=1), anti-E (n=1), anti-E+c (n=1), and anti-Le_a (n=4), with three unidentified results in the NaCl/Enzyme identification. However, these samples were only weakly reactive in LISS/Coombs identification, and most of them remained unidentified with the LISS/Coombs method. These antibodies have a chance of being missed in screening if only LISS/Coombs methods were used in antibody screening. Thus, the LISS/Coombs method for screening could fail to detect some significant unexpected antibodies.

For antibody identification, there are significant differences between the LISS/Coombs and NaCl/Enzyme methods (Table 3). The NaCl/Enzyme method showed more strong reactions and could detect more antibodies. Some samples showed positive in the LISS/Coombs screening were positive only in the NaCl/Enzyme identification. And, it has the advantage of discriminating antibodies when mixed antibodies were present. In addition, 11 samples were positive only in the NaCl/Enzyme identification, although these were positive in both screening methods. So the use of NaCl/Enzyme method is essential for antibody identification. However, the anti-Fy_a and anti-Xg_a antibodies were found on screening and were identified only by the LISS/Coombs method. Therefore, NaCl/Enzyme method should be used together with LISS/Coombs method.

In the present study, the NaCl/Enzyme method showed about threefold higher detection rates, especially of Rh antibodies in screening, and higher exact identification rates and discrimination power for mixed antibodies. In conclusion, simultaneous LISS/Coombs and NaCl/Enzyme testing is useful for antibody screening and identification because the NaCl/Enzyme method can detect and identify many significant antibodies that would be overlooked if only the LISS/Coombs method was used, although a high proportion of antibodies found in NaCl/Enzyme screening are insignificant and reflect nonspecific reactions.

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