A comparison of a new affinity column system with a conventional tube LISS-antiglobulin test for antibody detection

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A recently introduced system for antibody detection (ReACT™) consists of affinity columns (AFC) that contain protein A and protein G-coated agarose. We compared the ReACT™ system to a conventional tube low-ionic-strength saline antiglobulin test (LISS-AGT). We selected 100 LISS-AGT positive samples with clinically important and benign antibodies of varying strengths and 130 LISS-AGT negative samples to evaluate by the AFC method. AFC tests were positive with all 84 clinically important antibodies, including 36 antibodies that reacted ≤ 1+ at LISS-AGT (0% falsely negative). Eleven of 16 (69%) clinically benign antibodies reacted by AFC. Five samples (2 anti-Sd[a], 2 anti-I, and 1 inconclusive) were negative by AFC. AFC tests were negative with all 130 samples that were negative by LISS-AGT (0% falsely positive). The AFC method showed results comparable with results obtained with a conventional tube LISS-AGT for detection of clinically important antibodies. Some unwanted, clinically benign antibodies may not be detected by the AFC method. ***Immunohematology* 1999;15:75–77.**

Key Words: affinity column, microcolumn, antibody detection

Microcolumns containing agarose coated with protein A and protein G have been developed for red blood cell (RBC) antibody detection tests. In contrast to other microcolumn systems that trap agglutinated RBCs in an immunologically inert matrix, the ReACT™ (Red Cell Affinity Column Technology) system (Gamma Biologicals, Houston, TX) depends on the affinity adherence of IgG-sensitized RBCs to an immunoreactive matrix.

We compared the affinity column (AFC) method to a conventional tube low-ionic-strength antiglobulin test (LISS-AGT) for antibody detection.

Materials and Methods

The LISS-AGT was performed using a LISS additive solution (Ortho-Clinical Diagnostics, Raritan, NJ). Antibody screening tests consisted of mixing 2 drops of serum or plasma, 2 drops of LISS, and one drop of 3% to 4% reagent RBCs, incubating for 15 minutes at 37°C, and then converting to the antiglobulin test using monospecific anti-IgG (Organon-Teknika, Durham, NC). Agglutination was graded from 0 to 4+. Antibody identification tests were performed using the LISS method according to the guidelines in the American Association of Blood Banks *Technical Manual*.[1] Using results obtained with the standard tube LISS-AGT, we selected 100 serum or plasma samples with positive antibody screening tests that, on further testing, contained antibodies of varying reaction strengths and clinical importance (Table 1), and 130 samples that had negative antibody screening tests. These samples were stored at –80°C until testing was performed by the AFC method.

Table 1. Antibodies selected for evaluation of the affinity column system (categorized by LISS antiglobulin test [LISS-AGT] reaction strength and clinical importance)

Antibodies	LISS-AGT agglutination category		
Clinically important	No.	≤1+	2+ to 4+
Rh system	45	15	30
K	20	8	12
MNSs system	8	5	3
Kidd system	4	2	2
Duffy system	3	3	0
Other	4	3	1
Total	84	36	48
Clinically benign	No.	≤ 1+	2+ to 4+
Lewis system	4	4	0
Cold agglutinin	3	3	0
Knops system	2	2	0
Anti-Sd[a]	2	2	0
Inconclusive*	5	5	0
Total	16	16	0

*Antiglobulin reactivity of no apparent specificity; all common clinically significant antibodies excluded

The affinity column system (ReACT™) was obtained from Gamma Biologicals. This method consists of adding one drop of serum or plasma and one drop of LISS-suspended (0.8%) RBCs to each column, incubating at 37°C

for 15 minutes, followed by centrifuging for 3 minutes. Reactions were read as either positive or negative. A test that showed RBCs remaining above or in the immunoreactive matrix was considered positive. A test that showed all of the RBCs at the bottom of the immunoreactive matrix was considered negative.

Results

Antibody screening tests by the AFC method were performed on a total of 230 samples: 100 with antibodies of varying reaction strengths and clinical importance and 130 samples that did not contain atypical antibodies. The results of these tests are summarized in Table 2. All 84 clinically important antibodies, including 36 antibodies that were ≤ 1+, were detected using the AFC method (0% falsely negative). Eleven of 16 clinically benign antibodies also were detected by the same test. Five of the 16 benign antibodies, anti-Sd^a (2), anti-I (2), and inconclusive (1), were not detected using the AFC method. The 130 inert samples were nonreactive by the AFC method (0% falsely positive).

Table 2. Results using the affinity column system for antibody detection

	LISS-AGT Antibody Categories			
	Clinically important		Clinically benign	
Results	≤ 1+	2+ to 4+	≤ 1+	Negative
Affinity column positive*	36	48	11	0
Affinity column negative	0	0	5	130
Total	36	48	16	130

*Affinity column test results were interpreted as either positive or negative

Discussion

Microcolumn systems offer several advantages over standard test tube methods.[2] In particular, reactions in microcolumns are stable and more reproducible than the typical agglutination reactions of test tube techniques.[2]

A microcolumn system containing gel particles (the gel test) and antiglobulin serum for antibody detection was developed by Lapierre et al.[3] in 1985, and several studies have demonstrated the utility of this method.[2-5] In the gel test, the gel acts as a molecular sieve that separates agglutinated from unagglutinated RBCs.

In contrast to the gel test system, the microcolumn system used in the present study contains proteins A and G covalently coupled to the agarose.[6] Proteins A and G are bacterial proteins that bind specifically to the Fc portion of IgG molecules. Protein A binds to IgG1, IgG2, and IgG4 subclasses but does not bind to IgG3. Protein G binds to all four IgG subclasses. Coupling these proteins to agarose creates an immunoreactive matrix or an affinity column system. RBCs sensitized with IgG antibody will specifically adhere to the matrix, whereas unsensitized RBCs will pass through the matrix.

In this article, we compared the AFC system to our standard tube LISS-AGT for antibody detection. The study was designed to test the affinity column system with antibodies of varying strength and clinical importance and with samples that had negative antibody screening tests by a standard tube LISS-AGT. The AFC system reactions were relatively easy to read, and antibodies that reacted only weakly by the LISS-AGT were clearly discernable using the affinity columns. Although reactions by the affinity column system in our study were only graded as positive or negative, the strength of the reactions were consistent with the LISS tube method. Antibodies that reacted 3+ to 4+ by a LISS-AGT reacted strongly by the affinity column method with virtually all RBCs remaining above or in the immunoreactive matrix. Alternatively, antibodies that reacted only weakly by the LISS-AGT method generally reacted only weakly by the affinity column system, with only a small portion of the screening cells remaining above or within the matrix. Our results indicated that the AFC system is comparable with our LISS tube method for the detection of clinically significant antibodies. The study also showed that some unwanted or clinically benign antibodies (e.g., anti-Sd^a) may not be demonstrable using the AFC system. This finding is not surprising because many of the clinically benign antibodies are IgM; the AFC system is designed to be specific for IgG alone.[6]

The AFC system offers the advantages of microcolumn system technology and appears to be comparable in sensitivity and specificity with a standard tube LISS-AGT for the detection of clinically important antibodies.

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