The gel test: use in the identification of unexpected antibodies to blood group antigens

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The IgG GEL test was compared with the LISS tube test (Löw and Messeter's low-ionic-strength saline) for antibody identification. The suitability of red blood cells (RBCs) pretreated with ficin, dithiothreitol (DTT), or chloroquine diphosphate (CDP) also was assessed for use in the GEL test. In addition, time-in-motion studies were performed comparing GEL (12 panels per batch) with polyethylene glycol (PEG) tube tests (3 panels per batch). In 57 antibody identification studies, there were 63 GEL+ LISS+, 2 GEL+ LISS–, and 6 GEL–LISS+ antibodies. Among the GEL+ LISS+ antibodies were 19 that yielded stronger reactions in GEL than in LISS; by virtue of their specificity, 14 of these are considered potentially significant: D, 5 E, 2 e, 2 Jk^a , 2 S, K, and Fy^a. There were 38 antibodies that yielded equivalent results by both methods, including 31 that are considered potentially significant. Of six antibodies with significantly greater reactivity in LISS, there were three anti-Rh and three that are considered harmless with respect to transfusion management. The two GEL+ LISS– antibodies (anti-Jk^b) were potentially significant. GEL–LISS+ reactions involved only harmless antibodies. Of the 50 antibodies of potential significance, GEL yielded equivalent or superior results in 47 (94%) instances. Additionally, GEL failed to detect 6 of 21 harmless antibodies. Expected results were obtained with normal serum or plasma and antibodies of known specificity in tests with RBCs treated with ficin, DTT, or CDP. Hands-on-time required for each GEL panel was 2 to 2½ minutes compared with 12 minutes for PEG. These data document the suitability of GEL for use in antibody identification studies. ***Immunohematology* 1998;14:59–62.**

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New technologies such as the IgG GEL test[1] require crossover validation before implementation. Currently, there are no published reports from U.S. facilities that compare the use of GEL with conventional tube technologies in the identification of unexpected antibodies.

Results of our crossover validation study for antibody detection by GEL have been described elsewhere.[2] In this present report, we document our findings when comparing GEL with our routine antibody identification procedure that is based on the low-ionic-strength saline (LISS) procedure of Löw and Messeter.[3] We also present data establishing the suitability of GEL for use with both enzyme-treated and chemically modified red blood cells (RBCs), as well as the results of time-in-motion studies comparing GEL with polyethylene glycol (PEG) tube tests.[4]

Materials and Methods

An ID-Micro Typing System™ for performing the IgG GEL test was obtained from Ortho Diagnostic Systems Inc. (Raritan, NJ). GEL tests were set up and graded as recommended by the manufacturer. However, in our laboratory, reactions in GEL were read using an illuminated view box, and doubtful reactions were evaluated using a hand-held magnification lens.

LISS tests were performed by incubating one volume (0.1 mL) of 2% reagent RBCs in LISS (Löw and Messeter's formulation,[3] Immucor, Inc., Norcross, GA) with an equal volume of patient's serum. The reactants were not warmed to 37°C before mixing. Incubation was for 15 minutes at 37°C, after which the tests were centrifuged and examined for agglutination and hemolysis. The RBCs were then washed × 4 with sodium chloride before testing with polyclonal (rabbit) anti-IgG (Ortho). Reactions were read macroscopically with the aid of an illuminated concave mirror and graded and scored as described previously.[5] All negative antiglobulin tests were verified with IgG-coated RBCs (Immucor).

For comparative antibody identification studies between GEL and LISS, group O reagent RBCs were either from Gamma Biologicals, Houston, TX; Immucor; or Organon Teknika Corp., Durham, NC. The same RBC samples were used for both GEL and LISS. Samples used for GEL testing were predominantly from EDTA-anticoagulated blood (to reduce interference from fibrin); serum was used for LISS. Comparative studies were performed on samples selected at random on patients with positive LISS tests for unexpected antibodies encountered during pretransfusion testing. Technologists performing identification studies by GEL were not privy to the findings by LISS.

Results by GEL were compared with those by LISS. Discrepancies were evaluated by repeat testing by both methods; some were investigated by additional tube tests using room-temperature incubation, polyspecific antiglobulin serum, ficin-treated RBCs, or PEG.[4,5]

In other tests, selected donor RBCs were treated with either ficin, dithiothreitol (DTT), or chloroquine diphosphate (CDP), as described elsewhere.[5] These were prepared and used by GEL according to the manufacturer's recommendations for antibody detection with untreated RBCs. Sera or plasmas tested against the treated RBCs were from our routine caseload; some had been stored frozen at –70°C.

For time-in-motion studies, GEL panels were done in batches of 12. Both hands-on and throughput times were measured[6] and compared with those required to perform three panels by PEG. The latter were done with commercially available PeG® (Gamma), as described by the manufacturer. Four technologists performed a total of 10 timings for each batch method. Each panel consisted of 11 reagent RBC samples and an autologous control. Times that are constant for both methods (e.g., sample centrifugation) were not measured.

Results

The results of GEL and LISS tests on 71 antibodies from 57 samples are compared in Table 1. There were 63 GEL+ LISS+, 2 GEL+ LISS–, and 6 GEL– LISS+ antibodies. Among the GEL+ LISS+ antibodies were 38 GEL = LISS, 19 GEL > LISS, and 6 GEL < LISS antibodies (where = denotes no observed difference, > or < denotes an observed difference of at least one grade of reactivity between the two methods). By virtue of their specificity, 31 of 38 GEL = LISS antibodies are considered potentially significant with respect to transfusion management. Of 19 GEL > LISS antibodies, 14 are potentially significant, whereas 3 of 6 GEL < LISS antibodies are clinically important. The 2 GEL + LISS– antibodies (anti-Jk^b) were both potentially significant. The remaining GEL > LISS, GEL < LISS, and the 6 GEL– LISS+ antibodies are considered harmless with respect to transfusion management or of uncertain clinical significance. There were 8 antibodies (3 E, M, Le^a, Le^b, 2 cold-auto) that reacted only as direct agglutinins in LISS; of these, the 3 anti-E and the Le^a were detected by GEL.

Table 1. Comparison of antibody identification studies by GEL and by LISS

Results	Wanted*	Unwanted*	Total
GEL = LISS†	3 D, 3 C, c, 2 E, 2 cE, f, C^w 7 Jk^a; 2 S; 5 K; 4 Fy^a	RhIG‡; M; cold-auto 4 warm-auto	38
GEL > LISS§	D, 5 E(3‖), 2 e 2 Jk^a; 2 S; K; Fy^a	M; Le^a‖; 2 warm-auto NOID¶	19
GEL < LISS**	2 E, D	M; P_1; cold-auto	6
GEL+ LISS–	2 Jk^b		2
GEL– LISS+		M‖; N; Le^a, Le^b‖ 2 cold-auto‖	6
Total	50	21	71

* Presumed significance based on specificity and derived from clinical experience with respect to transfusion of incompatible blood
† Reactions by GEL equal to those by LISS-IgG
‡ Anti-D passively acquired from Rh immune globulin
§ Reactions by GEL stronger than those in LISS-IgG by at least one graded reaction strength
‖ Antibodies detected by LISS as direct agglutinins at 37°C; nonreactive with anti-IgG
¶ No identification; both positive and negative tests seen; no discernible specificity
**Reactions by GEL weaker than those in LISS-IgG by at least one graded reaction strength

Table 2 compares the results of GEL tests with untreated and ficin-treated RBCs. The anticipated enhancement of reactivity was seen with Rh and Kidd system antibodies, and the expected loss of reactivity was observed with antibodies to protease-sensitive antigens (e.g., Fy, MNS). The reactivity of other antibodies in GEL was variably affected by pretreatment of RBCs with ficin, consistent with their reported reactivity in tube tests.

Table 2. Effect of ficin treatment of red blood cells* on blood group antibody reactivity in GEL tests

Anti-	n†	Not Treated	Ficin-Treated	Anti-	n†	Not Treated	Ficin-Treated
D	2	10‡	12	Fy^a	3§	8.7	0
C	2	11	12	Fy^b	2§	9	0
c	3	10.7	12	M	1§	8	0
E	3	5.3	11.7	N	1§	9	0
e	1	10	12	S	2§	10.5	1
Jk^a	3	6.7	9.7	s	2§	7.5	0
Jk^b	2	6.5	10	Ch	2	8	0
Jk3	4	7.5	9.5	Rg	1	3	0
K	3	7.3	9.7	JMH	1	8	0
k	1	8	10	Yt^a	2	9	6.5
Kp^b	1	10	12	Lu^b	1	8	5
Js^b	1	10	10	Lu17	1	5	4

* Each antibody tested against same single-dose red blood cell sample, both treated and untreated, except as otherwise indicated. § For antibodies to high-prevalence antigens, presumed double-dose red blood cells were used
† Number of samples tested
‡ Values represent average reaction score[5]
§ For these antibodies, single-dose untreated and apparent double-dose ficin-treated red blood cells were used

In other tests, we evaluated the specificity of GEL with ficin-treated RBCs (data not shown in Table 2). Of 70 serum or plasma samples from patients with negative screening tests for unexpected antibodies by LISS tube technique, 61 were nonreactive with ficin-treated RBCs by GEL. The nine reactive samples included three that contained enzyme-dependent panagglutinins; one example each of anti-HI, -E, and -Le^a; two that exhibited no discernible specificity; and one sample that yielded a spuri-

ously positive reaction.

The results of tests with DTT- and CDP-treated RBCs by GEL are shown in Table 3. DTT-treated RBCs gave the expected negative or weaker reactions with antibodies to antigens known to be sensitive to thiol reagents (e.g., Kell system, JMH, Yt^a). All other specificities studied reacted equally well with both untreated and DTT-treated RBCs in GEL. Three examples of anti-Bg were reactive in GEL with untreated RBCs and gave the anticipated negative results with CDP-treated RBCs. All other antibodies tested in GEL reacted with CDP-treated RBCs to the same degree as untreated RBCs. No unwanted positive tests were encountered with either DTT-treated RBCs (n = 64) or CDP-treated RBCs (n = 22; data not shown in Table 3).

Table 3. Effect of chemical treatment of red blood cells* on blood group antibody reactivity in GEL tests

Anti-	Not Treated	DTT†-Treated	CDP‡-Treated	Anti-	Not Treated	DTT-Treated	CDP-Treated
K	8§,5,9‖	0,0,0¶	8	JMH	8	0	8
k	8	0	8	Yt^a	8,10	0,0	8,9
Kp^b	10	4	11	Fy^a	8	8	8
Js^b	10	0	9	Fy^b	8	8	8
D	10	10	11	M	8	8	8
C	12	11	10	N	9	9	NT**
c	10	8	8	S	10	10	10
E	11	11	11	s	4	4	3
e	10	10	10	Lu^b	8	8	8
Jk^a	88	8	Lu17	5	5	5	
Jk^b	58	5	Ch	8,8	9,8	8,8	
Jk3	8,8,8,8	10,8,6,8	6	Rg	3	3	3
				Bg	4,4,3	NT	0,0,0

* Each antibody tested against same single-dose red blood cell sample, both treated and untreated, except as otherwise indicated.¶ For antibodies to high-prevalence antigens, presumed double-dose red blood cells were used
† Dithiothreitol
‡ Chloroquine diphosphate
§ Values represent average reaction score [5]
‖ Multiple results represent test scores obtained with different examples of these antibodies; underlining indicates samples to be compared
¶ For three anti-K, single-dose untreated and apparent double-dose DTT-treated red blood cells were used
** Not tested

Table 4 shows the results of time-in-motion studies. Hands-on time for GEL was 25 minutes (batch of 12 panels) compared with 37 minutes for PEG (batch of 3 panels). There was no difference in the throughput times for the two batch methods. However, the time taken to prepare reagent RBCs for use in GEL is excluded from Table 4, because cell preparation for GEL can be performed once daily rather than for each batch. In our studies we found that it takes 5 minutes to prepare sufficient RBCs to perform up to five batches of 12 panels by GEL per day. Given this information, and using the batch numbers described, the hands-on time required for each GEL panel equals 2 to 2½ minutes, compared with 12 minutes for PEG.

Table 4. Results of time-in-motion* studies comparing GEL with PEG

GEL Tests—12 panels†	TIME‡	PEG Tube Tests—3 panels
label cards	1	label tubes
	2	
prepare patient RBCs × 12	3	
	4	
	5	prepare patient RBCs × 3
	6	
add panel RBCs × 12	7	add panel RBCs × 3
	8	
	9	
	10	add patient RBCs × 3
add patient RBCs × 12	11	add serum for Patient 1
	12	add PEG to tests for Patient 1
	13	incubate tests for Patient 1
add patient sera × 12	14	
	15	
	16	
	17	add serum for Patient 2
place cards in incubator	18	add PEG to tests for Patient 2
	19	incubate tests for Patient 2
	20	
	21	
	22	add serum for Patient 3
	23	add PEG to tests for Patient 3
	24	incubate tests for Patient 3
	25	
	26	
	27	
	28	
	29	wash tests for Patient 1§
	30	
	31	
	32	
centrifuge cards	33	add AHG‖ to tests for Patient 1 and spin
	34	wash tests for Patient 2
	35	read tests for Patient 1
	36	
	37	
	38	add AHG to tests for Patient 2 and spin
	39	wash tests for Patient 3
	40	read tests for Patient 2
	41	
	42	
	43	add AHG to tests for Patient 3 and spin
read and record reactions	44	
	45	read tests for Patient 3
	46	
	47	
	48	
	49	

* Shading denotes hands-on time
† Excludes time taken to prepare panel red blood cells for GEL (see text)
‡ Average of 10 separate timings
§ PEG tube tests washed × 3
‖ Anti-human globulin
¶ Time taken to read PEG panels includes time taken to verify negative tests with IgG-coated red blood cells

Discussion

Data presented in this article demonstrate the suitability of GEL for use in antibody identification studies. Potentially significant antibodies are clearly identified, falsely positive results are not obtained with samples yielding negative antibody detection tests, and the causes of positive antibody detection tests are readily elucidated. Moreover, GEL detected two potentially significant antibodies not reactive by LISS and failed to detect six harmless antibodies that were reactive in LISS. Although LISS gave stronger reactions than GEL with three potentially significant Rh antibodies, nine harmless antibodies were either solely reactive in LISS or gave stronger reactions in LISS than in GEL. According to these results, GEL is equal to or better than LISS for routine antibody identification studies.

GEL is also suitable for use with RBCs pretreated with ficin, DTT, or CDP, as evidenced by (1) the absence of change in reactivity with antibodies to antigens that are not affected by these treatments; (2) the expected enhanced reactions of ficin-treated RBCs with Rh and Jk antibodies; (3) the expected loss of reactivity with antibodies to antigens known to be denatured by ficin, DTT, or CDP; and (4) the fact that treated RBCs did not yield unexpected reactions in GEL to any greater extent than we have experienced in tube tests. In addition, tests used in the investigation of serologic problems, such as inhibition studies and tests of eluates and adsorbed serum, can be performed successfully.[7] Our experience in these and similar regards is limited (data not presented), but RBCs recovered from samples stored in glycerol at –70°C[5] can be utilized in GEL. Eluates prepared by organic solvents or by the freeze–thaw technique[5] cannot be tested by GEL because of contamination with hemoglobin. Furthermore, the sensitivity of GEL for IgG autoantibodies may necessitate more extensive adsorption than is required for some tube tests.

The results of time-in-motion studies clearly demonstrate the increase in productivity that can be achieved using GEL technology for antibody identification. Although the workload in most laboratories does not permit testing in batches as large as those we used, it should be appreciated that batch testing of three samples by GEL entails about one-third of the hands-on time required to test the same samples by PEG.

Gel column technology provides other advantages over traditional tube testing.[1,7] The stability of reactions allows for repeated review of test results by multiple technologists. GEL reactions can be graded more objectively than those in tube tests, and the use of standardized volumes of reactants enhances test reproducibility among laboratory staff. All of these advantages serve to facilitate the interpretation of serologic observations encountered in antibody identification studies.

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