Implementation of gel column technology, including comparative testing of Ortho ID-MTS with standard polyethylene glycol tube tests

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With the intent to increase laboratory efficiency and according to the Clinical Laboratory Improvement Act of 1988 (CLIA '88), a parallel testing program comparing traditional tube technology with the gel system technology was undertaken. Test tube indirect antiglobulin tests were performed using polyethylene glycol (PEG) as the antibody enhancement medium. Gel (GEL) column technology used the ID-Micro Typing System™, using predispensed anti-IgG and low-ionic-strength saline for antibody enhancement. Tests were performed as described in the manufacturer's guidelines and the current edition of the *Technical Manual of the American Association of Blood Banks*. Testing included antibody detection, antibody identification, direct antiglobulin tests (DATs), antigen phenotyping (K, Fy^a, Fy^b, S, and s), and elution studies. These procedures were evaluated for sensitivity, specificity, and efficiency. Sixty-six samples that had been tested for antibody activity by PEG tube techniques were evaluated by GEL. These samples included 49 that were nonreactive and 17 with a positive antibody detection test. Within the latter were 19 antibodies, 17 with specificities considered to be clinically significant and 2 usually considered clinically insignificant for red cell transfusion. GEL was nonreactive with the 49 PEG negative samples as well as with the 2 samples containing insignificant antibody. All 17 antibodies of probable clinical significance were detected. Antibody identification studies were performed on these latter samples, with GEL results consistent with PEG tube results in all cases. Concordant results were obtained with 10 of 10 DATs (7 negative, 3 positive), all 77 antigen phenotyping tests (37 negative, 40 positive), and the 6 parallel elution studies (4 negative, 2 positive). GEL testing was found to be comparable or better when compared with PEG tube testing in all procedures evaluated. ***Immunohematology* 1998;14:72–74.**

Key Words: ID-MTS, PEG-IAT, DAT, GEL

Gel technology is a relatively new system for performing direct and indirect antiglobulin testing.[1] The gel test was introduced in Europe in 1988[1,2] and has been used in the United States since 1995.

The gel test uses the principle of controlled centrifugation of red blood cells (RBCs) through a dextranacrylamide gel and predispensed reagent in a specifically designed microtube. Precise volumes of serum or plasma, and/or RBCs are dispensed into the reaction chamber of the microtube. If necessary, the gel card, consisting of six microtubes, is incubated, then centrifuged. Agglutinated RBCs become trapped in or above the gel. Unagglutinated RBCs move through the gel and form a pellet in the bottom of the microtube.

With the intent to increase laboratory efficiency and according to the requirements of the Clinical Laboratory Improvement Act of 1988[3] (CLIA '88), a parallel testing program comparing traditional tube technology with gel technology was undertaken. This study compares standard tube testing using polyethylene glycol[4] (PEG) as an enhancement medium with results obtained with the gel (GEL) technology of ID-Micro Typing Systems™ (Ortho Diagnostics Systems Inc., Raritan, NJ). The methods were evaluated for specificity, sensitivity, and efficiency for the following tests: antibody detection, antibody identification, direct antiglobulin tests (DATs), antigen phenotyping (K, Fy^a, Fy^b, S, and s), and elution studies. Tests were performed as described in the manufacturer's guidelines and the 11th edition of the *Technical Manual of the American Association of Blood Banks*,[5] with some minor modification as noted in the tests for antigen phenotyping and elution studies.

Materials and Methods

Samples

Serum and EDTA plasma specimens submitted to the blood bank laboratory for routine pretransfusion testing were evaluated by the indirect antiglobulin test (IAT). Samples were tested in parallel by standard PEG tube tests (PeG®, Gamma Biologicals, Houston, TX), and ID-Micro Typing System™ gel column with predispensed anti-IgG (Ortho).

Parallel DATs by each method were performed on

RBCs obtained from either EDTA-anticoagulated samples or cord whole blood samples using both polyspecific anti-human globulin (AHG) and anti-IgG (Ortho). RBCs for selected parallel antigen typings (K, Fy^a, Fy^b, S, and s) were obtained from pretransfusion samples or from commercially prepared sources.

RBCs for elution studies were collected in EDTA anticoagulant and prepared using ELU-KIT II™ (Gamma) according to the manufacturer's guidelines.

Standard tube testing

PEG tube tests for antibody detection and identification were performed according to the manufacturer's guidelines. Specifically, two drops of serum or plasma and one drop of reagent RBCs were mixed. These were centrifuged immediately and observed for agglutination and hemolysis. Two drops of PEG were then added. Following incubation for 10 minutes at 37°C, the tubes were again visually inspected for hemolysis, followed by the IAT using anti-IgG (Ortho) and both macroscopic and microscopic readings. Negative tests were controlled by the use of IgG-sensitized RBCs (Ortho).

DATs were performed with polyspecific AHG, according to the manufacturer's guidelines. Negative tests by immediate spin were incubated at room temperature for 5 minutes, followed by recentrifugation. Both macroscopic and microscopic readings for agglutination were performed; negative tests were controlled by the use of IgG-sensitized RBCs. Positive samples were further tested with anti-IgG to differentiate IgG sensitization from complement sensitization.

Selected antigen phenotyping was performed using commercially prepared antisera, according to the manufacturer's directions.

Eluates were tested according to the manufacturer's package insert, including recommended modifications to enhance sensitivity for antibody detection whenever the DAT of the RBCs was weakly reactive (increased incubation time up to 30 minutes). The final supernatant was run as a control to ensure adequate washing. Anti-IgG was used for the IAT.

Gel column method

All tests were performed using ID-MTS Anti-IgG Cards. Antibody detection, antibody identification, and DATs were performed according to manufacturer's instructions. Antigen typing and eluates were tested according to the antibody detection procedure, substituting 25μL of antisera or eluate in place of serum.

Test protocol

Samples were coded prior to testing. The technologist performing subsequent testing did not know previous test results. Two technologists performed all tests and grading of agglutination was standardized for each method. All results were compared at the antiglobulin phase.

Results

Antibody detection

Parallel antibody detection was performed on 66 samples (Table 1). Forty-nine were found to be negative and 16 were positive by both methods. One sample was positive with tube testing but negative with GEL.

Table 1. Results of parallel antibody detection tests

Results	PEG	GEL
Negative	49	51
Positive: clinically significant*	17	17
Positive: clinically insignificant†	2	0

* Specificities associated with hemolytic transfusion reactions or decreased survival of transfused antigen positive RBCs [5]

† Specificities not known to be, or only rarely, associated with hemolytic transfusion reactions or decreased survival of transfused antigen positive RBCs [5]

Antibody identification

Antibody identification was performed on all 17 samples (1 sample contained 3 antibodies), yielding a positive antibody screen. Seventeen antibodies (2 D, 3 E, c, 4 K, Fy^a, Jk^a, Jk:3, S, s, and 2 M) of a specificity and reactivity suggestive of clinical relevance with respect to the selection of homologous donor RBC units for transfusion were reactive in PEG and GEL. Two antibodies (Le^a and N) that are usually considered clinically insignificant with regard to RBC transfusion were detected with tube tests but not with GEL. GEL and tube testing displayed comparable reactivity with antibodies of specificity suggestive of clinical relevance (Table 2).

Direct antiglobulin tests

All three cord blood samples were negative by both methods (including room-temperature incubated tube tests), as were four of the EDTA samples. The three positive samples yielded comparable results with both polyspecific AHG and anti-IgG: 3+, 1+, and 2+, respectively. When tested by GEL, these samples were 4+, 2+, and 2+, respectively.

Selected antigen phenotyping

All five commercial antisera (K, Fy^a, Fy^b, S, and s), were tested in parallel against a full reagent RBC panel and an

Table 2. Results of parallel antibody identification studies

Specificity	PEG	GEL
Fy^a	pos*	2–3+†
Jk^a	pos*	1–2+
K	1+‡	1+
S	pos*	2–3+
M	2+	±-3+
s	pos*	3+
D	2+	2–3+
K	2+	2–3+
E§	2+	2+
c§	1+	±–1+
N§	2+	0
M	2–3+	4+
K	2+	2–3+
E	3+	3–4+
K	2+	3+
E	3+	4+
D	mi+¶	±–2+
Le^a	2+	0
Jk:3	mi+¶	2+

* Frozen stored samples, graded strength not available
† Reaction strengths graded according to manufacturer's guidelines
‡ Reaction strengths graded according to previously published guidelines[5]
§ One sample contained three antibodies
¶ Microscopically positive only

antibody detection set. Identical results were obtained (Table 3). The RBCs of 7 patients, known to be alloimmunized, were tested by both methods for corresponding antigens (4 K, Fy^a, S, and s) and found to be negative.

Table 3. Results of parallel selected antigen phenotypings

Antisera	RBC antigen status	Tube test	GEL
K	Positive	3*	3
	Negative	15	15
Fy^a	Positive	7	7
	Negative	8	8
Fy^b	Positive	10	10
	Negative	4	4
S	Positive	6	6
	Negative	9	9
s	Positive	11	11
	Negative	4	4

*Number of samples tested for each result

Eluate testing

Eluates were prepared and tested from six positive DAT samples. Parallel testing was comparable in all studies. Four samples were nonreactive by each method. One sample contained antibody of indeterminate specificity that reacted 3+ with 14 of 14 reagent RBC samples in GEL and routine tube IAT testing. The final sample, obtained from a recently transfused patient, yielded anti-E, reactive 2+ by tube and 4+ in GEL.

Efficiency evaluation

During performance of the parallel testing by two technologists, other personnel were asked to make a subjective appraisal of gel technology with respect to simplicity of testing and increase in productivity. Although no formal data were determined, all training participants reported that, in their opinion, gel technology yielded a more simplified and efficient test system.

Discussion

When compared with standard tube testing, using PEG as the antibody enhancement reagent, ID-MTS gel system technology yielded several benefits:

- Comparable or enhanced sensitivity
- Increased specificity
- Increased efficiency
- Simplified testing procedures

These advantages were noted in all testing situations evaluated: antibody detection, antibody identification, DATs, antigen phenotyping, and elution studies.

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