Evaluation of a Modified IAT-Gel With Polyethyleneglycol (PEG) Addition Method for Red Blood Cell Antibody Identification

L. Gustafsson, A. Wikman, J. Lundahl*

Division of Clinical Immunology and Transfusion Medicine, Karolinska Hospital, Stockholm, Sweden

We aimed to evaluate whether a PEG-IAT method, modified for gel technique, could replace the two-stage enzyme method for routine identification of red blood cell (RBC) antibodies.

A total of 161 selected patient samples were analyzed with the two-stage enzyme tube technique and the PEG-IAT in gel techniques. In 65 samples, identical antibody results were obtained by the two methods. An additional 28 antibodies were identified by either the PEG-IAT gel method or the enzyme method. Twenty antibodies were identified by the PEG-IAT gel method only (12 Rh antibodies, four anti-Kell, two

anti-Fya, two anti-Jka), and eight antibodies by the enzyme method only (four Rh antibodies, one anti-K, one anti-Jka, two anti-Lea). The results in the remaining 68 samples were judged as nonspecific reactions, which were more common when the enzyme method was used.

We concluded that the PEG-IAT in gel method identifies more RBC antibodies with less nonspecific reactions than the two-stage enzyme technique. However, no single method detected all clinical significant RBC antibodies. *J. Clin. Lab. Anal.* 18:165–169, 2004. © 2004 Wiley-Liss, Inc.

Key words: PEG; IAT-gel; two-stage enzyme; RBC antibody identification

INTRODUCTION

Red blood cell (RBC) antibody identification is routinely done by using at least two different techniques. The most frequently used techniques are the indirect antiglobulin test (IAT) in gel or tube techniques and the enzyme technique. The enzyme method is a sensitive technique, especially when used to identify Rh-antibodies. A recognized disadvantage is the high proportion of nonspecific reactions, which require follow-up investigations with uncertain clinical value. Moreover, the enzyme treatment causes the destruction of Duffy- and MNS-antigens and is also generally regarded as a time-consuming method (1).

Polyethylene glycol (PEG) is a water-soluble polymer that enhances the antigen–antibody reaction by reducing the Z-potential (2). The PEG-IAT technique has been shown to be sensitive and specific in RBC antibody detection (3–5). In addition, the PEG solution is easy to prepare and store. In recent years, the gel technique has been introduced for antibody screening (6). The method is easily standardized and has, in many countries, become the most common technique used in blood bank laboratories (7).

Given the advantages of the PEG-IAT and the gel techniques, we addressed the question as to whether a combination of these two techniques, the PEG-IAT in

gel method, could replace the two-stage enzyme method in routine RBC antibody identification. The study consisted of an initial comparison between a standard PEG-IAT method in tube and a modified PEG-IAT in gel technique. In the second part, we compared the modified PEG-IAT in gel technique with a two-stage enzyme method for RBC antibody identification.

MATERIAL AND METHODS

Red Blood Cells

The RBC were selected from phenotyped blood donors, frozen in glycerol, thawed, and thereafter washed four times with phosphate-buffered saline (PBS) (8). The RBC were prepared as 3% (tube test) and 0.8% (gel test) low ionic strength saline (LISS) suspensions in ID-CellStab (DiaMed, Cressier-sur-Morat, Switzerland), and stored for 21 and 28 days,

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*Correspondence to: Joachim Lundahl, MD, PhD, Division of Clinical Immunology and Transfusion Medicine, Karolinska Hospital, Building L2:01, S-17176 Stockholm, Sweden.

E-mail: joachim.lundahl@ks.se

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respectively, at +2 to +8°C. RBC were homozygous for a number of major antigens (Rh-system, Kidd, Duffy). A panel including RBC from 10 donors was used for antibody identification. The same panel was used for the gel, the tube, and the enzyme methods.

Sera

Large scale random serum samples (n = 39,750) from patients and donors were screened for irregular RBC antibodies with gel cards LISS/Coombs (-IgG, -C3d)

(DiaMed). In this test, 1,474 samples were judged as positive and subjected to further analysis. Routine antibody identification was primarily done with the IAT-gel test (see below). In 161 samples, the IAT-gel test generated inconclusive results, and these samples (n = 161) were subjected to subsequent analysis with the PEG-IAT in gel and the two-stage enzyme tube methods (see below).

Selected sera from patients and pregnant women with RBC antibodies of known specificity (Rh, Kell, Kidd, Duffy) were also used in the study. The identification was done with a combination of different IAT-gel and

Fig. 1. Flow chart showing the testing procedure for identification of RBC antibodies in patients, donors, and pregnant women using a combination of IAT-gel and two-stage enzyme tube techniques.

two-stage enzyme tube techniques. A flow chart for the testing procedure is presented in Fig. 1.

IAT Gel Method

To the upper well of the gel card microtubes (LISS/Coombs; DiaMed), 50 μ L of 0.8% RBC suspended in ID-CellStab and 25 μ L patient serum (or plasma) were added. The gel cards were thereafter placed in the incubator at 37°C for 15 min before centrifugation. The reaction was read macroscopically and graded from 0 (negative) to 4+ (maximum reaction). This gradation scale was valid for all techniques used in the present study.

Two-Stage Enzyme in Tube Method

RBC, frozen and thawed, was treated with 3% papain, according to a standard method (9). One drop of serum was incubated with one drop of papain-treated RBC at 37°C for 90 min. The reactions were thereafter read in microscope.

Polyethylene Glycol

PEG (MW 4000; Merck, Darmstadt, Germany) was solubilized in PBS to gain 10%, 20%, and 30% solutions. Solutions were kept at +4°C for 1 month.

PEG-IAT in Tube Method

Two drops of serum (or plasma), one drop 3% RBC suspended in ID-CellStab, and four drops of 20% PEG solution were mixed in a tube and incubated at 37°C for 15 min. After four washes in PBS, two drops anti-IgG (Gamma-Clone, Gamma Biologicals Inc., Houston, TX) were added to the dry cell button. After centrifugation, agglutination was read macroscopically.

PEG-IAT in Gel Method

One drop of serum (or plasma), two drops 0.8% RBC suspended in ID-CellStab, and two drops of 20% PEG solution were mixed in a tube and incubated at 37°C for 15 min. After one wash in PBS, one drop of the dry cell button was transferred to a gel card with specific anti-IgG (Anti-IgG ID Micro Typing; DiaMed). The gel card was then centrifuged and the agglutination was graded.

RESULTS

Selection of Optimal PEG Concentrations and Red Cell Suspension Volumes

In order to establish the optimal concentration of the PEG solution, four sera with known antibody specificity (one anti-C^w, one anti-E, one anti-D+C, one anti-

Jkb+E) were tested against a selection of representative test cells (Table 1). The PEG-IAT in gel method was used. Results were congruent between the 20% and the 30% PEG solutions, whereas in the 10% PEG solution, one anti-D+C was considered negative against one test cell. A gradual increase in the reaction strength was noted in one anti-E, but was considered positive in all three concentrations used. No false positive reactions were noted with the highest concentration.

To select optimal volume of the 20% PEG suspension, we compared two droplets with four droplets. Three sera with known antibody specificity (one anti-C, two anti-K) were titrated against selected test cell. As shown in Table 2, only minor differences between the two volumes were observed. Based on these results, we selected two droplets 20% PEG combined with two droplets of the RBC suspension, to be used in our PEG-IAT in gel method.

Comparison Between the PEG-IAT in Gel and the PEG-IAT in Tube Methods

To evaluate whether the PEG-IAT in tube method could be replaced by the PEG-IAT in gel method, we

TABLE 1. Selection of the optimal PEG concentration

Antibody	Test cell	PEG 10%	PEG 20%	PEG 30%
Anti-C ^w	CC ^w De/CDe	Negative	Negative	Negative
	CDE/cDE	Negative	Negative	Negative
Anti-E	CDE/CDe	Negative	Negative	Negative
	CDE/cDE	1+	2+	3+
Anti-D+C	CDE/cDE	3+	3+	3+
	Cde/Cde	Negative	2+	3+
	Cde/cde	Negative	Negative	Negative
Anti-Jk ^b +E	Jk(a-b+)	ND	2+	2+
	E-e+			
	Jk(a+b-)	ND	3+	3+
	E+e-			
	Jk(a+b+)	ND		3+
	E-e+		Negative	Negative

ND, not done.

TABLE 2. Selection of the optimal volume of PEG suspension

Titer	Anti-C		Anti-K*		Anti-K*	
	PEGg 2 dr	PEGg 4 dr	PEGg 2 dr	PEGg 4 dr	PEGg 2 dr	PEGg 4 dr
1:2	4+	3+	2+	2+	4+	4+
1:4	3+	3+	1+	-	4+	3+
1:8	2+	2+	-	-	4+	3+
1:16	-	1+	-	-	3+	3+
1:32	-	-	-	-	2+	2+
1:64	-	-	-	-	-	-

*Two different anti-K sera.

TABLE 3. Comparison between the PEG-IAT in tube and the PEG-IAT in gel methods

Antibody	PEG-IAT in tube	PEG-IAT in gel
-C ^w	1+	3+
-C ^{w*}	2+	Negative
-C	1+	1+
-E	3+	3+
-E	3+	3+
-E	2+	2+
-D	1+	2+
-D	Negative	Negative
-D	Negative	Negative
-Fy ^a	3+	4+
-K	4+	2+
-K	2+	3+
-Fy ^b (1:32)**	3+	3+
-D(1:64) [†]	3+	3+

*Anti-C^w is considered to be of IgM-type.

**Anti-Fy^b (Human serum; Immucor, Norcross, GA).

[†]Anti-D (Human monoclonal blend; Immucor).

used both methods to test 14 sera with known antibody specificity. According to Table 3, one anti-C^w was considered negative in the PEG-IAT in gel method but positive in the PEG-IAT in tube method. The overall accumulated antibody score was equal for both methods, 101 points each.

Validation of the PEG-IAT in Gel Method

The PEG-IAT in gel method and the two-stage enzyme in tube method were run in parallel to identify RBC antibodies in selected sera (n = 161). These sera had given inconclusive results when tested by the IAT in gel method. In 65 samples (40%), identical results were obtained with both methods. An additional 28 antibodies were identified by the PEG-IAT in gel or the enzyme methods. Twenty of these antibodies were identified by the PEG-IAT in gel method only (12 Rh antibodies, four anti-Kell, two anti-Fya, two anti-Jka) and eight antibodies by the enzyme method only (four Rh antibodies, one anti-K, one anti-Jka, two anti-Lea) (Table 4). Nonspecific reactions were more common in the enzyme method (n = 50), than in the PEG-IAT in gel method (n = 18).

DISCUSSION

In the present study, we demonstrated that the PEG-IAT in gel method identified more RBC antibodies with less nonspecific reactions than the two-stage enzyme in tube technique. However, no single method detected all clinical significant RBC antibodies.

In the first part of our study, we compared a standard PEG-IAT in tube method with a modified PEG-IAT in

TABLE 4. Number of antibodies and antibody mixtures detected by PEG-IAT in gel, or the two-stage enzyme, techniques only

Antibody/antibodies			
Specificity	Number	PEG-IAT in gel	Two-stage enzyme
Anti-D	3	1 id	2 id
Anti-E	8	6 id	2 id
Anti-C	2	2 id	—
Anti-c	1	1 id	—
Autoanti-e	1	1 id	—
Anti-K	4	3 id + 1 (one cell positive)	1 id
Anti-Fy ^a	2	2 id	—
Anti-Jk ^a	3	2 id + 1 (homozygous cell)	1 id,
Anti-Le ^b	2	Negative*	2 id
Anti-c,-E	1	1 id	—
Anti-E,-K	1	1 id	Only anti-E id
Non-specific	68	18	50

*Anti-Le^b is of IgM-type, and only anti-IgG is added to the gel card. Id, identified.

gel method. In a comparison of 14 antibodies with different specificities, the overall accumulated score was equal for both methods. However, with the PEG-IAT in gel method, one anti-C^w was not detected. We presumed that this antibody was of IgM-class and therefore escaped detection by the gel technique but was detected by the PEG-IAT in tube method due to the additional spin step. Because the gel technique was an easily standardized technique, we preferred to use the gel technique in the second part of the study, where we compared the PEG-IAT in gel method with the two-stage enzyme in tube method used in our laboratory. In 65 out of 161 selected sera, identical results were obtained with both methods. In 20 sera, additional antibodies were detected by the PEG-IAT in gel method, and in eight sera, additional antibodies were detected by the enzyme method. One proposed advantage with the enzyme technique is the identification of Rh antibodies, but in the present study we could not find any indications of weaker reactions with Rh-antibodies by the PEG-IAT in gel method. However, it must be emphasized that our 161 samples were selected based on inconclusive results given by the IAT in gel method, and that other criteria for selection might generate other results.

The additional antibodies identified by the PEG-IAT in gel method were all antibodies with specificities usually considered to have clinical significance, while two anti-Leb were identified by the enzyme method. These “enzyme only” antibodies have previously been reported to be clinically insignificant in blood transfusion and maternal screening (10,11).

Several factors favor the use of the PEG-IAT in gel method. Nonspecific reactions were 2.8 times more common in the enzyme method than in the PEG-IAT in gel method. Nonspecific reactions can lead to unnecessary investigations and can, apart from extra costs, cause delay in transfusion procedures and operation schedules (1). In the PEG-IAT in gel method, the incubation period is shortened compared to the enzyme technique, which reduces the analysis time. The use of the gel technique also facilitates the interpretation of results due to the macroscopic reading compared to the microscopic reading when the enzyme method is used. In addition, the enzyme treatment leads to the destruction of Duffy- and MNS-antigens, while incubation with PEG does not modify major RBC antigens.

To summarize, the PEG-IAT in gel method is easy to implement, is not time-consuming, and it enables identification of clinical significant antibodies with a low frequency of nonspecific reactions. Therefore, we propose that the PEG-IAT in gel method may replace the two-stage enzyme in tube method for routine RBC antibody identification.

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