A Comparative Study of Three Techniques for Eluting Red Cell Antibodies

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Abstract

Sixty-seven eluates obtained from the heat, ether and acid elution techniques were tested with the specific red blood cells (RBCs) and were compared according to their reactivities using the indirect antiglobulin test (IAT). It was found that the ether elution technique was superior in eluting Rh antibodies except for anti-e while the acid elution technique was superior in eluting Miltenberger (Mi^a) antibodies ($P < 0.05$). The heat elution technique gave the lowest reactivity among the three techniques.

In conclusion, the reactivities of the eluates obtained from the acid elution technique were overall comparable to those from the ether elution technique. The acid elution technique is practical for routine use in most blood banks because it is less time consuming and reduces the risk of exposing hazardous chemicals.

Elution of antibodies from red blood cells (RBCs) is used mainly in investigating the positive results of the direct antiglobulin test (DAT) and for the separation of mixtures of antibodies in order to identify them by testing with a panel of phenotyped group O RBCs. Elution can also be used in combination with the absorption technique for confirmation of weak antigens on RBCs and in the preparation of typing reagents[1]. A variety of elution techniques for eluting antibody from sensitized RBCs have been described such as heat, ether, xylene, chloroform, acid, ultrasound and microwave [1-6]. Ether, xylene and chloroform elution techniques involve the use of flammables or carcinogenic reagents. The heat and ether elution techniques are time-consuming. The acid elution method is commonly used because it yields consistent results and no hazardous chemicals are required.

In this study, we compared the reactivity of the eluates obtained from heat, ether and acid elution techniques.

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MATERIAL AND METHOD

Sensitization of RBCs

Selected DAT-negative group O RBCs were obtained from the anticoagulated specimens of the Department of Transfusion Medicine, Siriraj Hospital, Bangkok, Thailand. They were phenotyped for C, c, D, E, e, Jk^a, Jk^b and Mi^a and subsequently incubated with corresponding human derived antiserum obtained from transfused patients at 37°C for 1 hour in a water bath with intermittent mixing. Following incubation, the sensitized RBCs were washed 5 times manually with normal saline, the last washed supernatant was saved for parallel testing with the eluate. We ascertained the efficiency of the antibody coating by performing a DAT with broad spectrum antiglobulin serum (DiaClon Coombs, Dia Med AG, Switzerland).

Heat elution[1]

Equal volumes of 1.0 ml of sensitized washed packed RBCs and 1.0 ml of normal saline were mixed in a 13x100-mm test tube. The tube was placed at 56°C (water bath) for 10 minutes with frequent agitation. The RBC suspension was immediately centrifuged at 1,000 g for 1 minute in a tabletop centrifuge. The eluate was transferred into a clean test tube for further testing.

Ether elution[1]

Equal volumes of 1.0 ml of sensitized washed packed RBCs, 1.0 ml of normal saline and 1.0 ml of ether were mixed in a 13x100-mm test tube. The tube was corked and agitated vigorously for 1-2 minutes. The rubber cork was removed and the tube was placed at 56°C (water bath) for 10 minutes with frequent agitation. The tube was centrifuged at 1,000 g for 5 minutes. The eluate was transferred into a clean test tube for further testing.

Acid elution

Acid elution was performed as indicated on the package of the Dia Cidel elution kit (Dia Med AG, Switzerland)[7]. The sensitized RBCs were washed once with normal saline and 4 times with Dia Cidel working wash solution. Part of the last washed supernatant was kept for parallel testing with the eluate. Equal volumes of 1.0 ml of washed packed RBCs and 1.0 ml of Dia Cidel elution solution were mixed. The RBC suspension was centrifuged immediately at 1,000 g for 1 minute. The eluate was transferred into a clean test tube. Five drops of Dia Cidel buffer solution were added into the eluate, and mixed well. A blue color appeared indicating that a neutral *p*H 6.5-7.5 was reached. If the blue color was not obtained, more buffer was added 1 drop at a time while mixing. The eluate was centrifuged at 1,000 g for 1 minute to remove residual cells.

Determination of reactivity of eluates

The heat, ether and acid eluates were tested with specific group O RBCs using the standard low ionic strength saline indirect antiglobulin test (LISSIAT)[1]. The titer of each eluate, which gave a positive result for the IAT was determined by testing serial two-fold dilutions of eluate against selected RBC samples. The grading of agglutination reactions were scored as 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 0 for 4+, 3+s (s=strong), 3+, 2+s, 2+, 2+w (w=weak), 1+s, 1+, 1+w, w+ (macro), w+ (micro) and negative, respectively[8]. In addition, the IAT results of each eluate were compared with the DAT results of sensitized RBCs in order to demonstrate the evidence of elution by each technique.

Statistical Analysis

The Pearson correlation test was used to analyse the statistical difference between the mean values of the agglutination scores of the eluates from three elution techniques. A P-value of less than 0.05 was considered statistically significant difference[9].

RESULT

Sixty-seven samples of the eluates obtained from heat, ether and acid techniques were compared for their reactivities. The mean scores of the titer of the heat, ether and acid eluates are shown in Table 1. We found that the efficiency of the ether elution technique was comparable or better than the acid elution technique especially for eluting Rh (except anti-e) antibodies ($P<0.05$) and Kidd (Jk^b) antibodies ($P>0.05$). The acid elution technique was superior for eluting Miltenberger (Mi^a) antibodies ($P<0.05$) and Kidd (Jk^a) antibodies ($P>0.05$). In this study the heat elution technique was the least sensitive technique for eluting of all antibodies while the acid elution technique required the least time for preparation of the eluates. The evidence of elution by each technique was suggested by the observed reduction in DAT reaction strength (Table 2).

Table 1. Comparison of the agglutination scores of the eluates obtained from heat, ether and acid elution techniques.

Antibody	Number	Agglutination scores (mean ± SD)			P - value
		heat	ether	acid	
Anti-D	6	18.33±8.55	52.33±14.32	32.17±13.1	P1= 0.001, P2= 0.014
Anti-C	2	4.00±1.41	16.00±11.31	14.50±16.26	Not tested
Anti-C + e	3	4.00±1.73	18.00±19.50	30.67±9.29	Not tested
Anti-c	7	4.43±6.13	22.00±22.94	15.00±9.22	P1= 0.035, P2= 0.529
Anti-E	13	20.00±12.95	57.62±21.15	33.92±21.58	P1= 0.004, P2= 0.002
Anti-e	6	8.83±10.80	10.50±10.90	18.67±14.73	P1= 0.080, P2= 0.043
Anti-Jk^a	6	3.33±3.20	17.83±12.35	21.00±12.49	P1= 0.802, P2= 0.582
Anti-Jk^b	7	7.43±7.16	32.29±19.64	17.71±10.00	P1= 0.450, P2= 0.692
Anti-Mi^a	17	18.29±23.3	11.18±18.74	26.65±25.64	P1= 0.001, P2= 0.000

P1 = Comparison between heat and ether eluates,
P2 = Comparison between ether and acid eluates

Table 2. Comparison of Coombs' reduction of eluates obtained from heat, ether and acid elution techniques indicated by grading of agglutnation reactions.

Antibody	Number	DAT*	Eluates (IAT)		
			heat	ether	acid
Anti-D	6	3+	2+	3+	3+
Anti C	2	1+s	W+	1+	1+s
Anti C+e	3	2+	1+	1+	2+
Anti c	7	2+	w+	2+	2+
Anti E	13	2+s	2+	3+s	3+
Anti-e	6	2+	1+	1+	2+
Anti-Jk^a	6	1+s	w+	1+	1+s
Anti-Jk^b	7	2+	1+	2+	2+
Anti-Mi^a	17	2+	1+s	1+	2+*

*DAT of coated cells

DISCUSSION

A variety of methods have been described for eluting antibodies from sensitized RBCs. In this study, 67 eluates obtained from the heat, ether and acid elution techniques were compared for their reactivities with the specific RBCs. Our results confirmed the premise that the acid and ether elution techniques were significantly better for eluting of warm reactive antibodies (Rh and Kidd) than the heat elution technique, which should be restricted to the investigation of ABO hemolytic disease of the newborn(1,2). Furthermore, the acid elution technique was found to be superior in eluting anti-Mi^a which is one of the common antibodies found in the Thai population(10).

Since ether, xylene and chloroform are organic solvents and the techniques involved are time consuming, the acid elution technique which is safe, fast and sensitive can replace hazardous chemicals in routine laboratory work. Moreover, the acid eluate color was blue and clear while the heat and ether eluates were cherry-colored which is difficult to read and interpret the agglutination reactions.

In conclusion, the acid elution technique was able to elute most common RBC antibodies from *in vitro* DAT - positive cells for most common RBC antibodies. Results obtained with this technique were overall comparable to those obtained from the ether technique and it is more practical for most blood banks.

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การศึกษาเปรียบเทียบวิธีการ elute แอนติบอดีย์บนผิวเม็ดเลือดแดง

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ได้ทำการศึกษาเปรียบเทียบ eluates ที่เตรียมได้จากวิธี heat, ether และ acid จำนวน 67 ตัวอย่าง ทำการทดสอบ eluates ที่ได้กับเม็ดเลือดแดงที่จำเพาะกับแอนติบอดีย์แต่ละชนิดโดยใช้ indirect antiglobulin tests และตรวจหาระดับของแอนติบอดีย์ใน eluate พบว่าวิธี ether ให้ผลดีในการ elute แอนติบอดีย์ในระบบ Rh ยกเว้น anti-e ส่วนวิธี acid ให้ผลดีกับ anti-Mi^a ($P < 0.05$) ส่วน eluate ที่ได้จากวิธี heat พบว่ามีความแรงของปฏิกิริยาน้อยที่สุด

จากการศึกษานี้แสดงว่า การ elute แอนติบอดีย์โดยใช้วิธี acid ให้ผลดีกับการ elution แอนติบอดีย์ทุกระบบที่นำมาศึกษา แม้ว่าในบางระบบจะมีประสิทธิภาพด้อยกว่าวิธี ether แต่ไม่มีนัยสำคัญทางสถิติ นอกจากนี้วิธี acid มีข้อดี คือ ใช้เวลาน้อยและลดความเสี่ยงของผู้ปฏิบัติงานในการที่ต้องสัมผัสกับสารเคมีอันตรายดังที่ใช้ในวิธี ether จึงเหมาะในการนำมาใช้ในงานธนาคารเลือด

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