Z Rechtsmed (1985) 95:163–166

Zeitschrift für
Rechtsmedizin

A Simple Method for Purification of Anti-A and Anti-B Antibodies Using Glutaraldehyde-fixed Human Red Blood Cells

Y. Katsumata and M. Sato

Department of Legal Medicine, Nagoya University School of Medicine, Nagoya 466, Japan

Summary. A simple method for purification of anti-A and anti-B antibodies using glutaraldehyde-fixed human erythrocytes is described. Specific antibodies were first absorbed with the corresponding cells, then eluted by heating at 53°–55°C for 15 min. The method is simple and highly efficient with a fair recovery of 15.6%–34.4%.

Key words: Antibody purification, glutaraldehyde – Anti-A and anti-B antibodies, purification

Zusammenfassung. Es wird eine einfache Methode zur Reinigung von Anti-A- und Anti-B-Antikörpern mittels Glutaraldehyd-fixierten Human-Erythrozyten beschrieben. Spezifische Antikörper wurden zuerst mit den entsprechenden Zellen absorbiert und daraufhin bei 53°–55°C für 15 min eluiert. Die Methode ist einfach und sehr leistungsfähig mit Ausbeuten zwischen 15,6 und 34,4%.

Schlüsselwörter: Antikörper-Reinigung, Glutaraldehyd – Anti-A- und Anti-B-Antikörper, Reinigung

Recently, we devised a new method for detection of blood type A or B substances in body fluids using a sandwich type of enzyme-linked immunosorbent assay (ELISA) [1]. However, an overnight incubation step is included, and the blank values are somewhat high in the method because commercially available anti-A or anti-B is used without further purification. Thus, purification of the specific antibodies is necessary for shortening the time required and for lowering the blank values in the method. The purification of anti-A or anti-B antibodies is usually made by affinity chromatography [2–5] requiring purified blood-group substances.

In the present study, a simple method for purification of anti-A or anti-B using human type A or type B erythrocytes for the affinity carrier.

Offprint requests to: Y. Katsumata (address see above)

Materials and Methods

Materials

Human type A and type B blood specimens were kindly supplied by the clinical laboratory of Nagoya University Hospital after necessary determinations had been made. Anti-A and anti-B antisera of human source were obtained from Ortho Diagnostic Systems, K.K. (Tokyo, Japan) and anti-A antiserum originated in rabbits and anti-B antiserum originated in goats from Tokyo Standard Serum Ltd. (Tokyo, Japan). Centriflo was obtained from Amicon Corp. (Denver, CO, USA).

Fixation of Human Type A or Type B Red Blood Cells with Glutaraldehyde

1. Human type A or type B red blood cells are freed from plasma.
2. To 1 ml of the pellet, 5 ml of 2.5% glutaraldehyde in 10 m*M* phosphate-buffered saline (PBS), pH 7.6, is added and allowed to incubate for 1 h at room temperature.
3. The cells are washed five times with PBS.
4. To the pellet, 5 ml of 1 *M* Tris-HCl (pH 8.0) is added and allowed to incubate for 2 h at room temperature.
5. The cells are washed five times with PBS. Washing is further repeated if the pH of the supernatant is not close to that of PBS.
6. The cells are resuspended in equal volumes of PBS containing 0.1% bovine serum albumin and 0.1% sodium azide, and stored at 4°C.

Procedure of the Present Purification Method

1. To 2 ml of the antiserum, an appropriate volume of the corresponding cell suspension (50%) is added and allowed to incubate overnight at 4°C.
2. The cells were washed 6–7 times with PBS. Washing is further repeated if the absorbance at 280 nm of the supernatant is higher than 0.05.
3. The cells are resuspended in 4 ml of PBS and incubated for 15 min at 53°–55°C.
4. After incubation is completed, the suspension is immediately centrifuged at 3,000 rpm for 1 min, and the supernatant is collected.
5. Steps 3 and 4 are repeated, and the supernatants are combined.
6. The supernatant is concentrated to the original volume by Centriflo, and the agglutinin titer and the protein concentration are determined.

Results and Discussion

Commercially available anti-B of goat source was absorbed with various volumes of human type-B erythrocytes with or without fixation by glutaraldehyde (Table 1). Glutaraldehyde-fixed cells absorbed the corresponding antibodies much more efficiently than unfixed cells. Formation of highly packed aggregates with unfixed erythrocytes may inhibit further interaction of the type-B determinants on the surface of the cells with anti-B antibodies. On the other hand, fixed cells form very loose frameworks into which antibody molecules can easily penetrate.

The anti-B antibodies absorbed to glutaraldehyde-fixed type-B erythrocytes were eluted by heating the cells at 53°–55°C for 15 min as shown in Table 2. The recovery of the specific antibodies by the absorption-elution procedure was around 30%, which was comparable to other methods using purified antigen-conjugated sepharose [5].

Absorption of other preparations of anti-A or anti-B by glutaraldehyde-fixed erythrocytes of the corresponding blood types was investigated (Table 3). Although volumes of fixed cells required for efficient absorption of the corre-

Table 1. Absorption of goat anti-B by human type B red cells

Volume of red cell suspension[b] (μl)	Titer of the antiserum[a]	
	Unfixed cells	Glutaraldehyde-fixed cells
0	256	256
50	128	64
100	64	32
150	64	16
200	64	16
250	64	1
300	64	0

[a] 2 ml of the antiserum was used
[b] 50% red cell suspension was used

Table 2. Elution of the bound anti-B from glutaraldehyde-fixed red cells[a]

Volume of PBS added for elution (ml)	Titer of the eluted antibody			Recovery (%)
	1st elution	2nd elution	3rd elution	
2	64	16	4	32.8
4	32	8	2	32.8
8	16	4	2	34.4

[a] 200 μl of the fixed cells reacted with 2 ml of the anti-B used

Table 3. Absorption of the corresponding antibodies by glutaraldehyde-fixed type A or type B red cells

Volume of red cell suspension[b] (μl)	Titer of the antiserum[a]		
	Human anti-A	Human anti-B	Rabbit anti-A
0	128	256	128
50	128	32	8
100	64	8	4
150	32	4	2
200	8	2	1
250	8	1	1
300	8	1	0

[a] 1 ml of the antiserum was used
[b] 50% red cell suspension was used

sponding antibodies varied with preparations, different lots of the same kind of antisera, i.e., different lots of anti-A of human source, showed similar resluts. Thus, the most appropriate ratio of the cell suspension (50%) to antiserum should be determined in each kind of antisera; the ratios of 0.2, 0.1, 0.05, and 0.1 were applied to human anti-A, human anti-B, rabbit anti-A, and goat anti-B, respectively, in the present study.

Table 4. Purification of various anti-A and anti-B sera by the present method

Antiserum		Titer	Protein concentration (mg/ml)	Minimum hemagglutinating dose[a] (μg/ml)	Recovery (%)
Human anti-A	original	256	36.0	140	—
	purified	128	0.071	0.55	29.0
Human anti-B	original	256	32.8	130	—
	purified	128	0.060	0.47	15.6
Rabbit anti-A	original	256	13.5	53	—
	purified	64	0.056	0.88	15.6
Goat anti-B	original	256	11.5	45	—
	purified	128	0.058	0.45	26.7

[a] Protein concentrations of antisera divided by their titers

Specific anti-A or anti-B antibodies in four kinds of antisera were purified by the present method (Table 4). Highly purified anti-A or anti-B antisera were obtained from all preparations tested; minimum hemagglutinating doses were 0.45–0.88 μg/ml. Ago and Tsuganezawa [5] reported the purification of anti-A allohemagglutinin by affinity chromatography using purified A-substance-conjugated Sepharose 4B, and the minimum hemagglutinating doses were 7–27 μg/ml. Kaplan and Kabat [6] reported the purification of anti-A and anti-B by absorption and elution from insoluble blood group substances of corresponding blood types, and showed minimum hemagglutinating doses of 0.07–0.28 μgN/ml, i.e., 0.44–1.75 μg protein/ml. Their method requires 7 days of incubation for the absorption step as well as purification of blood group substances. On the other hand, the present method yielded highly purified anti-A and anti-B in 2 days without requiring purification of blood group substances. Furthermore, this method is applicable to purification of other antibodies having binding sites on red blood cells, such as anti-human erythrocytes.

References

1. Katsumata Y, Sato K, Tsutsumi H, Yada S (1984) A novel method for ABO grouping of mixed stains of saliva using enzyme-linked immunosorbent assay (ELISA). Acta Criminol Jpn 50:167–172
2. Tsuganezawa O (1977) Affinity chromatography for the specific purification of human serum and *Dolichos bifrorus* anti-A hemagglutinins. Jpn J Legal Med 31:255–268
3. Iida R, Ago K, Tsuganezawa O (1983) Immunosorbents for the purification of MN blood group system antibodies. Jpn J Legal Med 37:88–94
4. Harris-Smith PW, Fletcher SM (1983) Affinity-purified *Ulex europaeus* lectin: preparation and serological characteristics. J Forensic Sci Soc 23:225–231
5. Ago K, Tsuganezawa O (1984) Studies on the purification of anti-A allohemagglutinin by affinity chromatography. I. Comparative efficiency of several eluting methods. Jpn J Legal Med 38:49–58
6. Kaplan ME, Kabat EA (1966) Studies on human antibodies. IV. Purification and properties of anti-A and anti-B obtained by absorption and elution from insoluble blood group substances. J Exp Med 123:1061–1081

Received June 18, 1985