Detection of AB Antigen in Blood Stain Using Enzyme-Linked Immunosorbent Assay

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AOKI, Y., FUNAYAMA, M. and SAGISAKA, K. Detection of AB Antigen in Blood Stain Using Enzyme-Linked Immunosorbent Assay. Tohoku J. exp. Med., 1987, 152 (3), 277-281 — A new method for detection of AB antigen from blood stain using enzyme-linked immunosorbent assay (ELISA) is described. Flat-bottomed-wells of polystylen plate coated with human anti-B or rabbit anti-A were sensitized with AB antigen which was extracted from blood stain with 1% octyl-glucopyranoside in 0.1 M phosphate buffer pH 8.0. Mouse monoclonal anti-A or anti-B, and peroxidase conjugated anti-mouse immunoglobulin were added to the wells, respectively. Subsequently, the substrate was dropped into the wells, and the absorbance of the solution was measured. By this method, we could distinguish AB group blood stain from the mixed stain of A and B group bloods. When rabbit antiserum was used as the first antibody, differentiation between these antigens was unsuccessful presumably because of non-specific adsorption.

To detect ABH blood group antigens of blood and body fluid stains, the mixed agglutination or the absorption-elution method is commonly used. When the stains are contaminated with blood or body fluid of two or more blood groups, these methods, however, sometimes fail to determine the blood group correctly. It is generally difficult to determine whether or not an AB group antigen is contained in the blood stains judged as AB group which were suspected to be mixed stains. In this paper, we describe a new method to detect AB antigen from blood stain using ELISA.

MATERIALS AND METHOD

Antisera. Anti-B serum of human origin was purchased from Ortho Diagnostic (Raritan, NJ, USA). Rabbit IgG anti-A was prepared as described previously (Sagisaka et al. 1981). In brief, A- rabbit was immunized intravenously with A group red cell and resulting antiserum was absorbed with O and B group red cells. The antiserum was partially purified by the ammonium sulfate precipitation method. Mouse monoclonal IgM anti-A (lot: 536A-2; titer 1: 2,560) and anti-B (lot: 5361B, A-1; titer 1: 2,560) were kindly provided by Prof. Ikemoto S., Laboratory of Human Biology, Jichi Medical School.

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Peroxidase conjugated goat anti-rabbit IgG was obtained from MBL Co. (Nagoya) and rabbit anti-mouse immunoglobulin from Dako Co. (Copenhagen, Denmark).

Specimens. AB group whole blood was dropped on a clean cotton cloth and dried, and stored for 1 week at room temperature. For mixed stain, equal volumes of A and B group whole blood were dropped on a cotton cloth respectively, and stored as mentioned above. One square centimeter of each stain was soaked in 0.5 ml of 1% octyl-glucopyranoside (Nakarai Chemicals, Tokyo) in 0.1 M phosphate buffer, pH 8.0, for 1 hr at room temperature. After doubling dilution with the same buffer, the extract was kept at 37°C for 1 hr.

ELISA. The wells of flat-bottomed microtiter plate (Dynatech, Sussex, England) were coated with 100 μl of 1:100 diluted human anti-B, 1:100 rabbit anti-A or 1:400 mouse anti-B in 50 mM sodium bicarbonate buffer pH 9.6. After incubation for 2 hr, the antibodies were removed, and 200 μl of 3% bovine serum albumin in phosphate buffered saline (BSA-PBS) was dropped on each well and allowed to stand for 30 min to block non-coated surface of the wells. The BSA-PBS was removed, and the wells were washed with PBS containing 0.05% tween 20 (tween-PBS) 2 times, 100 μl of diluted extracts of blood stains were then added to each well and incubated for 1 hr. The extracts were pipetted out by 4 times washing with tween-PBS, and 100 μl of the first antibody diluted with BSA-PBS was added as follows: Exp. 1) 1:100 diluted rabbit anti-A with BSA-PBS was added to the wells coated with human anti-B, Exp. 2) 1:400 diluted mouse anti-A to the wells coated with human anti-B, Exp. 3) 1:400 diluted mouse anti-B to the wells coated with rabbit anti-A, Exp. 4) 1:100 diluted rabbit anti-A to the wells coated with mouse anti-B. The first antibody was allowed to react for 2 hr. After 4 times washing with tween-PBS, 100 μl of 1:500 diluted enzyme-conjugated antibody corresponding to the first antibody was dropped on each well. After incubation for 2 hr, the wells were washed 4 times. Then, 200 μl of substrate solution (0.01% o-dianisidine and 0.002% hydrogen peroxide in 0.01 M phosphate buffer pH 6.0) was added followed by incubation for 30 min. At the end of the incubation, 40 μl of 1 N hydrogen chloride was added to the wells to stop the enzyme reaction, and the absorbance at 405 nm was measured with microplate photometer MTP-12 (Corona Electric Co., Katsuta).

Fig. 1. Procedures of ELISA.
Detection of AB Antigen

The procedures and antibodies used at each step are indicated in Fig. 1. All the procedures were performed at room temperature.

RESULTS AND DISCUSSION

In the previous paper (Sagisaka et al. 1983), a method to detect AB antigen from body fluid stains was reported. This was based on that A and B antigens of AB blood group are located in the same macromolecule as pointed out by Morgan and Watkins (1956). As ABH antigens of red blood cell are insoluble in water (Kabat 1956), the method was not adopted to blood stain.

Recently, ELISA which is regarded to be more sensitive than hemagglutination has been employed to detect blood group antigen (Harris-Smith and Fletcher

![Fig. 2. ELISA reactions of Exp. 1. Extract of AB group blood stain (○—○), extract of mixed stain of A and B group blood (●—●).](image)

![Fig. 3. ELISA reactions of Exp. 2. Extract of AB group blood stain (○—○), extract of mixed stain of A and B group blood (●—●).](image)
Katsumata et al. (1984) reported an ELISA method to detect AB antigen from mixed saliva stain using the same principle of our method (Sagisaka et al. 1983).

Harris-Smith and Fletcher (1984) reported that ABH antigens were soluble in octyl-glucopyranoside, which was ascertained in our laboratory (Aoki et al. 1986). As shown in Figs. 2, 3, 4 and 5, when mouse monoclonal anti-A or -B was used as the first antibody, clear differentiation of AB antigen from mixture of A and B antigens was observed. But when the extract diluted 1:16 at Exp. 3 or diluted 1:8 at Exp. 2 was examined, the difference between them became unclear. The extract diluted 1:8 or more did not show group specificity. It might be caused by low recovery of ABH antigens by the extraction with octyl-glucopyranoside.

Fig. 4. ELISA reactions of Exp. 3. Extract of AB group blood stain (○—○), extract of mixed stain of A and B group blood (●—●).

Fig. 5. ELISA reactions of Exp. 4. Extract of AB group blood stain (○—○), extract of mixed stain of A and B group blood (●—●).
Detection of AB Antigen

It was supported by our experiment that blood stains extracted repeatedly with octyl-glucopyranoside had yet potent blood group activity. However, in Exps 1 and 4 in which rabbit anti-A was used as the first antibody, the similar enzyme reactions at the end stage between AB antigen and the mixture of A and B ones were observed. It probably meant that the enzyme labeled anti-rabbit IgG had cross reactivity with the coated antibodies (mouse and human immunoglobulins) and the constituents of serum in the extract, and that the rabbit anti-A of which group specificity was confirmed by routine hemagglutination reacted with the extracted substances other than A antigen. Because of rather low group specific activity of the extract these non-specific reaction exerted much influence to the ELISA. Higher recovery and purification of the group-specific substance and strict-specificity of labeled antibody are necessary to identify the group correctly.

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References