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ABO blood grouping of saliva from mixed body fluids by sandwich methods using monoclonal antibodies to tissue specific epitopes on blood group substance in saliva

Akihiko Kimura[1], **Fuseo Matsumura**[2], **Ken-ichiro Sodesaki**[2], **and Tsutomu Tsuji**[1]

[1]Department of Legal Medicine, Wakayama Medical College, 27 Kyuban-chou, 640 Wakayama, Japan
[2]Department of Forensic Science, Saga Medical School, Saga, Japan

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Summary. In this paper methods for ABO blood grouping of saliva from mixed body fluids have been established. Monoclonal antibodies to tissue specific epitopes on blood group substances in saliva were used as solid phase antibodies to catch the blood group substances. ABO blood grouping of saliva could be performed by these methods without interference from other body fluids (eg. semen, vaginal secretion, urine, sweat and serum). At least 16,000 and 3,000 fold dilutions of secretor saliva were sufficient for ABO blood grouping by sandwich ELISA and sandwich absorption-elution test, respectively.

Key words: ABO blood grouping – Saliva – Mixed body fluids – Monoclonal antibody

Zusammenfassung. In dieser Untersuchung wurden Methoden zur ABO-Blutgruppenbestimmung an Speichel aus gemischten Körperflüssigkeiten etabliert. Monoklonale Antikörper zu gewebsspezifischen Epitopen für Blutgruppensubstanzen in Speichel wurden als „solid phase“ Antikörper benutzt, um Blutgruppensubstanzen zu binden. Die ABO-Blutgruppenbestimmung von Speichel konnte mit diesen Methoden ohne Interferenz mit anderen Körperflüssigkeiten (z.B. Sperma, Vaginalsekret, Urin, Schweiß und Serum) durchgeführt werden. Speichelverdünnungen von Sekretoren zwischen 3.000fach und 16.000fach waren ausreichend für die ABO-Blutgruppenbestimmung mit Hilfe von „Sandwich-ELISA“ und bzw. „Sandwich-Absorptions-Elutions-Test“.

Schlüsselwörter: ABO – Bestimmung – Speichel – gemischte Körperflüssigkeiten – monoklonale Antikörper

Offprint requests to: A. Kimura

Introduction

Blood grouping of an individual body fluid from a mixture of body fluids is an extremely important problem in medico-legal examinations, which is not yet fully resolved. If ABO blood group substances (BGSs) in body fluids possess tissue specific epitopes (TSEs), a sandwich method would be the most suitable procedure for resolving this problem. The specific antibody against TSE on BGS is necessary for this sandwich method, therefore monoclonal antibodies (mAbs) are the most suitable for this purpose. In a preceding paper we reported about mAbs (P4-2F, P4-5C) against TSE on BGS in saliva [1]. These mAbs were tissue and species specific and could be suitable reagents as the solid phase antibodies in a sandwich method.

In this report, we describe ABO blood grouping of saliva from mixed body fluids by the sandwich method using these anti-saliva TSE mAbs.

Materials and methods

Antibodies and lectins. Monoclonal antibodies (P4-2F, P4-5C) were prepared according to a previous paper [1]. Anti-A and -B serum (Human) were purchased from Ortho Diagnostic Systems (NJ, USA). Anti-H serum (Chicken) was a generous gift from the National Research Institute of Police Science. Horseradish peroxidase (HRP) conjugated anti-human Ig (goat) was obtained from CALTAG Laboratories (CA, USA).

Buffers

Dilution buffer: 10 m*M* *Tris*-HCl, pH7.4 containing 0.5 *M* NaCl, 0.2% Tween-20, 0.3% gelatin, 0.01% Thimerosal.

Washing buffer: 10 m*M* *Tris*-HCl, pH7.4 containing 0.5 *M* NaCl, 0.2% Tween-20.

Blocking buffer: 10 m*M* *Tris*-HCl, pH7.4 containing 0.15 *M* NaCl, 0.3% gelatin, 0.01% Thimerosal.

PBS: 10 m*M* phosphate buffered saline, pH7.4.

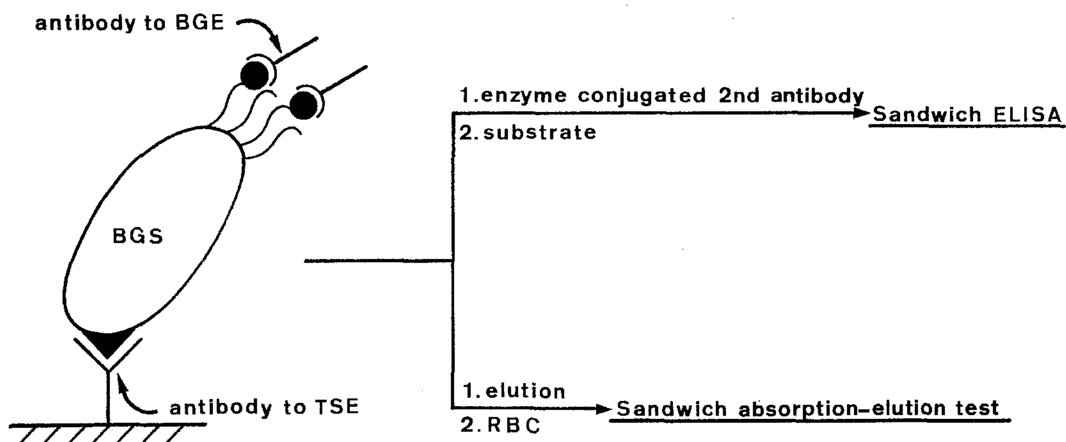

Fig. 1. Scheme of sandwich method

Sandwich ELISA. Wells of a flat bottom plastic plate (Nunc, Ill, USA) were coated with 50 µl of a 1000-fold dilution (PBS) of ascites of P4-2F or P4-5C and allowed to stand overnight at 4°C. The mAb coated plate was washed three times with PBS, blocked with blocking buffer and stored at 4°C until use. The plate was washed twice with washing buffer before use. Specimens of mixed body fluids (50 µl) were added to wells and incubated for 1 h at 37°C. The plate was washed five times with washing buffer and 50 µl of anti-A and anti-B (human, titer 1:10) were added. After 1 h incubation at room temperature, the plate was washed five times with washing buffer and incubated with 50 µl of HRP conjugated anti-human Igs (X2,000 dil) for 1 h at room temperature. 0-phenylenediamine was used as reagent for color development. Absorbance at 492 nm was measured by an EIA reader after addition of 50 µl 2 *N*. H_2SO_4 to each well.

Sandwich absorption-elution test. Preparation of plates coated with antibody and incubation with specimens were performed in the same way as described for sandwich ELISA. Then 50 µl of anti-A, anti-B (Human, titer 1:128) and anti-H (chicken, titer 1:128) serum was added to wells and incubated for 3 h at 37°C. The plate was washed five times with cold washing buffer, 30 µl of PBS was added and incubated for 15 min at 55°C. The appropriate indicator cells were added to each well and agitated for 30 min at room temperature. The hemagglutination was assessed microscopically.

Specimens. Native saliva and semen samples were diluted with dilution buffer. Stains and swabs of body fluids were extracted with dilution buffer by mechanical agitation.

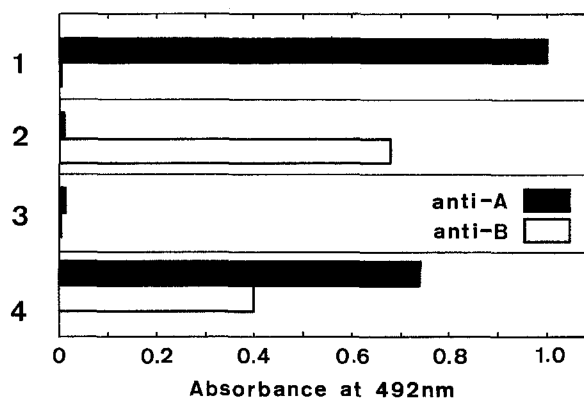

Fig. 2. ABO-blood grouping of secretor saliva mixed with secretor semen by sandwich ELISA. Specimens were prepared as follows: specimen 1, 25 µl of blood group A saliva (X250 dil) + 25 µl of blood group B semen (X250 dil); specimen 2, 25 µl of blood group B saliva (X250 dil) + 25 µl of blood group A semen (X250 dil); specimen 3, 25 µl of blood group O saliva (X250 dil) + 25 µl of blood group AB semen (X250 dil); specimen 4, 50 µl of blood group AB saliva (X500 dil). Procedures were as described in Materials and methods. The blank value was subtracted from the measurement

Results

The mAbs P4-2F and P4-5C recognize core proteins of BGS in saliva. These mAbs were used as the solid phase antibody to catch the BGS of saliva mixed with other body fluids. The detection of ABH epitopes on BGS which were caught by solid phase antibody, could be performed by ELISA and absorption-elution test (Fig. 1). The results of ABO blood grouping of 500-fold dilutions of secretor saliva mixed with 500-fold dilutions of secretor semen by sandwich ELISA using P4-5C are shown in Fig. 2. Although the blank values were relatively high (anti-A: 0.1~0.2, anti-B: 0.2~0.5), only ABO blood groups of saliva could be specifically detected without interference from semen. ABO blood groups of saliva mixed with other body fluids (vaginal secretion, urine, sweat, blood serum) were also detected specifically (data not shown). Similar results were obtained with P4-2F. In the conventional absorption-elution test, both the ABO blood groups from saliva and from the other body fluids were detected from these specimens (data not shown). Figure 3 shows the detection limits of ABO blood groups from secretor saliva by sandwich ELISA using P4-5C. The A and B epitopes of secretor saliva were detected to a dilution of at least 1.6×10^4. However, H epitopes were not detected satisfactorily by this method. The A and B epitopes of non-secretor saliva were detected only up to a dilution of 200~400 (data not shown).

Figure 4 shows the hemagglutination profile of a 50-fold dilution of secretor saliva mixed with a 50-fold dilution of secretor semen by the sandwich absorption-elution test using P4-2F. Only the ABO blood group epitopes (BGEs) of saliva were detected. The hemagglutination in positive wells was strong, so that they were easily seen with the naked eye. The ABO blood grouping of saliva mixed with other body fluids (vaginal secretion, sweat, urine, blood serum) was also specific.

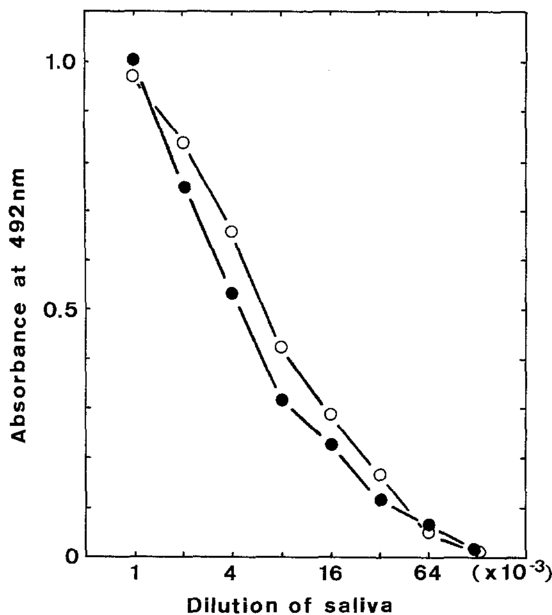

Fig. 3. The detection limits of ABO-blood groups from saliva by sandwich ELISA using P4-5C. Serial doubling dilutions of blood group A and B secretor saliva were used as specimens. Symbol (●) shows binding of anti-A antibody to dilutions of blood group A-saliva and symbol (○) shows binding of anti-B antibody to dilutions of blood group B-saliva. Procedures were as described in Materials and methods. The blank was subtracted from measurement.

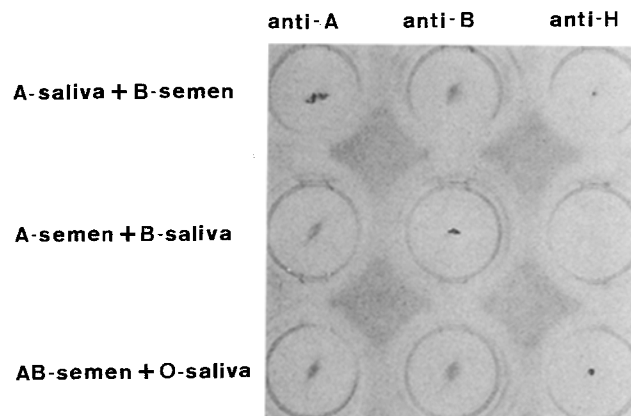

Fig. 4. ABO-blood grouping of saliva mixed with semen by sandwich absorption-elution test. Specimens were a mixture (1:1) of 50-fold dilution of secretor saliva and secretor semen. Procedures were as described in Materials and methods

In contrast to the ELISA, the ABH epitopes of saliva were detected up to a dilution of 1,600~3,200 by the sandwich absorption-elution test, irrespective of the secretor status. At least 1 mm^2 of stain of both secretor and non-secretor saliva was sufficient for blood grouping. Similar results were obtained with P4-5C (data not shown).

We made no errors in ABO blood grouping in fresh saliva samples and stains (A Se: $n = 8$, A se: $n = 2$, B Se: $n = 6$, B se: $n = 2$, 0 Se: $n = 8$, 0 se: $n = 2$, AB Se: $n = 4$, AB se: $n = 1$) examined in this study.

Discussion

In criminal case, stains often consist of a mixture of body fluids. Therefore, ABO blood grouping of a specific body fluid from mixed body fluids is an extremely important problem. In recent years, several attempts to perform ABO-blood grouping of semen from mixed body fluids have been reported. In these reports, polyclonal antibodies to seminal plasma were employed as capture antibodies in sandwich ELISA or in a immunodiffusion method [2, 3]. These antibodies were not specific for seminal BGS but only specific for seminal plasma. More recently, a method of ABO blood grouping of semen was reported, in which a polyclonal antibody to α_2-seminoglycoprotein (seminal ABO-BGS) was used as the capture antibody [4]. In our methods monoclonal antibodies to saliva BGS were employed which recognize the core protein of saliva BGS and are species and tissue specific.

As shown in Figs. 2 and 4, ABO blood grouping of saliva mixed with other body fluids could be performed by these methods. In ELISA, only A and B epitopes were identified, because a suitable combination of anti-H and the secondary antibody has not yet been found. H epitopes could be detected by the sandwich absorption-elution test, therefore, it must be possible to detect H epitopes by this method. As shown in Fig. 3, the detection limits of ABO-blood group epitopes using the sandwich ELISA were somewhat lower than ELISA or dot ELISA methods which were used to detect ABO blood groups from saliva with no contamination from other body fluids [5, 6]. The detection limits of ABO blood group epitopes from non-secretor saliva are much lower than secretor saliva using this methods. This phenomenon was also seen in dot ELISA [6]. The sensitivity of the sandwich absorption-elution test is lower than sandwich ELISA for the detection of ABO-BGEs from secretor saliva. However, it is comparable to conventional absorption-elution tests and is sufficient for routine use. This sandwich absorption-elution test can be performed without special reagents and equipment and there are only small differences in the detection limits between secretor and non-secretor saliva. Therefore, sandwich absorption-elution test is superior to sandwich ELISA in ABO-blood grouping of non-secretor saliva.

In this study, we have developed methods for ABO blood grouping of saliva mixed with other body fluids by sandwich methods using monoclonal antibodies to TSE on saliva BGS. A limited number of fresh saliva samples and stains have been examined, therefore, further investigations on saliva samples and aged stains should be carried out before the methods can be applied to medicolegal practice.

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