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A Sandwich Enzyme-Linked Immunosorbent Assay for ABO Blood Typing of Semen by Using Anti-p 84 Monoclonal Antibody as a Marker of Blood Group Substance in Semen*

REFERENCE: Sato I, Nakamura A, Ujiie K, Yukawa N, Najajima Y. A sandwich enzyme-linked immunosorbent assay for ABO blood typing of semen by using anti-p 84 monoclonal antibody as a marker of blood group substance in semen. J Forensic Sci 2000:45(4):795–800.

ABSTRACT: A blood group substance (BGS), a protein with ABH antigenic activity, was isolated from human seminal plasma and designated as p 84 (Sato, 1995) (1). We have developed a method for determining the ABO blood type of semen by performing a sandwich enzyme-linked immunosorbent assay (ELISA) in which p 84 is captured with an anti-p 84 monoclonal antibody, and evaluated the specificity and sensitivity of this method. Although BGS activity was detected in semen sensitively by this method, it was not detected in saliva, urine, breast milk, blood or vaginal secretions. Since the concentration of p 84 in semen was independent of the secretion status, the status can be determined as non-secretor when p 84 but not BGS activity was detected. To determine the stability of BGS activity on p 84, dried stains of semen on filter paper were kept at 4, 26, and 37°C for 8 months, 2 years and 1 month, respectively, and their BGS activities were examined. After 8 months at 4°C, over 60% of the original BGS activity was recovered from the stain. The activity could be detected even from a square as small as 0.25 by 0.25 cm. After 1 month at 37°C and 2 years at 26°C, 31 and 20% of the BGS activity, respectively, still remained. It could be detected from the pieces of 1.0 by 1.0 cm and 0.5 by 0.5 cm squares, kept for 1 month at 37°C and 2 years at 26°C, respectively. Finally, semen was mixed with saliva or blood at varying volumetric ratios and used for the sources of dried stains. The BGS activity of p 84 could be detected in the stains until the ratio between semen and saliva or blood reached 1:4. We conclude that this sandwich ELISA offers a more sensitive and specific method for determining the ABO blood type of semen samples obtained from sexual assault victims than existing methods, such as the conventional absorption-elution and classical hemagglutination-inhibition tests.

KEYWORDS: forensic science, p 84, ELISA, criminalistics, semen, ABO blood typing, sexual assault

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ABO blood typing of semen in forensic specimens obtained from sexual assault victims provides useful information not only for the initial criminal investigation, but also for screening sexual offenders. However, the more sensitive the method used to detect ABO (H) blood group substance (BGS), the more difficult it is to interpret the results, because almost all semen samples obtained under these circumstances are contaminated with the victim's body fluids, e.g., vaginal secretions, blood or saliva, which also bear BGS. It would be difficult to discern the ABO blood type of the semen alone. Although the traditional methods, including the hemagglutination-inhibition and adsorption-elution tests, and more sensitive methods, such as enzyme-linked immunosorbent assay (ELISA), enable the ABO blood group to be determined by analyzing human body fluids using serologic reagents containing monoclonal antibody (mAb) against the A, B and H antigens, these methods can not identify the individual BGS present in each body fluid. Recently, 2 BGSs in saliva and semen were separated from these fluids and used to produce an antibody, which can be used to determine the ABO blood type of each body fluid by carrying out a sandwich ELISA using anti-A, B and H mAbs following immunological capture of each BGS molecule (2 and 3). Although the forensic utility of this method has been demonstrated, the biological function and chemical structure of each BGS have not been characterized fully.

Sato (1995) detected a glycoprotein that bears ABH activity in human seminal plasma and designated it p 84 (1). Purified p 84 was found to be a single polypeptide chain with a relative molecular mass of 84 kDa, determined using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). When viable, motile sperm were incubated at 37°C in a suitable culture medium, p 84 was released from the sperm plasma membranes into the medium after the sperm had undergone capacitation, during which they lost their BGS (4). The mean concentration of p 84 in seminal plasma was 949 μg/mL by indirect ELISA using the anti-p 84 mAb. There was no significant difference in the concentration of p 84 between individuals who secreted (Se) or did not secrete (se). Additionally, an immunohistochemical study showed that p 84 was located in the cytoplasma of the inner layer of pseudostratified cuboidal epithelial cells of the seminal vesicles, but no immunoreactivity was found in the prostate (5). These data indicate that p 84 belongs to the family of sperm-coating antigen (SCA), and it is secreted from the seminal vesicles. These data also suggest that p 84 is distinct from 2 BGSs described above.

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In this study, we developed an ELISA for ABO blood typing of semen using ELISA plates precoated with an anti-p 84 mAb. We used this assay to search for BGS activity in various body fluids and found no such activity in saliva, urine, breast milk, blood or vaginal secretions. Subsequently, we determined the stability of the BGS activity on p 84 and the lower limit of detection of this BGS in semen contaminated with saliva and blood.

Materials and Methods

Specimens

Blood, saliva and urine and semen were obtained from volunteer donors. The ABO and Lewis blood type of each semen donor were determined by conventional hemagglutination test. Donors with blood group Le (a−b+) were categorized as Se, those with Le (a+b−) as se, and those with Le (a−b−) as undetermined (6). Breast milk was obtained from two lactating women, who were two to four months post-parturient. As for non-postcoital vaginal fluids, we used reconstituted fluids from our casework vaginal swabs that had given negative results for semen identification. Briefly, cotton vaginal swabs were immersed in 10-fold volume (relative to dry weight of the swabs) of 25 mM phosphate buffered-saline, pH 7.2 (PBS) at 4°C overnight and centrifuged at 5,000 × g to remove cellular debris. The protein concentration of the supernatant was then adjusted to 1 mg/mL with PBS. Each specimen was stored at −80°C until required for use. A portion of each specimen was blotted on a filter paper, allowed to dry to produce a stain and then stored under various environmental conditions for subsequent examination.

Reagents for Enzyme-Linked Immunosorbent Assay (ELISA)

Preparation of an anti-p 84 mAb of IgG1 subclass was described previously (5). Horseradish peroxidase (HRP)-conjugated goat antisera (anti-mouse IgG, anti-mouse IgM and anti-rabbit IgG) were from Tago Company (Burlingame, CA). Anti-A and anti-B mAbs were from Knickerbokker (Cromatest®, Barcelona, Spain), and the anti-H (O) mAb was from Chembiomed (Syntype®, Edmonton, Alberta, Canada).

Sandwich ELISA for ABO (H) Antigens on p 84

Each flat-bottomed well of an ELISA microtiter plate (Sumitomo Bakelite Co., Tokyo, Japan) was coated with the anti-p 84 (5 μg/mL in 100 mM sodium bicarbonate buffer, pH 9.6; 100 μL/well) at 4°C overnight. After being washed 3 times with PBS containing 0.05% (w/v) Tween-20, the well was incubated with 200 μL of gelatin (EIA grade, Bio-Rad Laboratories, Richmond, CA; 1% (w/v) in PBS) at 37°C for 1 h to block nonspecific binding sites. After being washed 3 times, the well was incubated with 100 μL of a sample appropriately diluted with PBS to capture p 84 molecule at room temperature (RT) for 2 h. After being washed 3 times, the well was incubated with 100 μL of the anti-A (1:200 in PBS), the anti-B (1:200 in PBS) or the anti-H (O) (1:40 in PBS) at RT for 3 h to target ABH antigens on the p 84 molecule. After being washed 3 times, the well was incubated with 100 μL of the HRP-conjugated anti-mouse IgM (1:2,000 in PBS) at RT for 1 h to detect the anti-ABO (H) antibodies. After being washed 5 times, the well was incubated with 100 μL of 4.6 mM o-phenylenediamine in 100 mM trishydroxymethylaminomethane-hydrochloric acid, pH 7.4, containing 0.03% (v/v) H_2O_2 at RT for 40 min in the dark to develop bound peroxidase activity. Finally, the enzyme activity was stopped by the addition of 100 μL of 2 N sulfuric acid, and the absorbance of each solution at 490 nm was measured by using an ImmunoReader NJ-2000 (Nippon Intermed Co., Tokyo, Japan). When the non-immune mouse IgG was used instead of the anti-p 84 mAb, the absorbance of the solution was usually 0.00 to 0.03. Therefore, an absorbance value of over 0.05 was construed as a positive reaction.

Indirect ELISA for p 84

The indirect ELISA for p 84 molecules (not for BGS activities on p 84) was performed as described previously (5). The anti-p 84 (5 μg/mL in PBS; 100 μL/well) was used as the primary antibody, and HRP-conjugate anti-mouse IgG (1:2,000 in PBS; 100 μL/well) as the secondary antibody. The other reagents and buffers were the same as those described above.

Confirmatory Test for Semen in Forensic Casework Samples

Stains, swabs and oral liquids from forensic casework samples were examined for acid phosphatase activity, p 30 (also known as γ-seminoprotein and prostate specific antigen), sperm and ABO blood type. Acid phosphatase activity was detected with SM test® (Ishizu Seiyaku Ltd., Osaka, Japan) and p 30 was measured by an indirect ELISA, basically as described above for p 84 and as reported previously (1), using anti-p 30 antiserum (Biomeda Co., CA, 1:2,000 in PBS) as the primary antibody and HRP-conjugated goat anti-rabbit IgG antiserum (Tago Co., 1: 2,000 in PBS) as the secondary antibody. Sperm was examined microscopically by staining with fuchsin acid and methylene blue. The ABO blood type was determined by the adsorption-inhibition test using anti-A and B mAbs (Cromatest®) and *Ulex europaeus* lectin I (Honen Co., Tokyo, Japan).

Results

Determination of the Various ABO Blood Types of Semen by the Sandwich ELISA

When semen samples, diluted 250-fold, from 54 individuals were subjected to the sandwich ELISA to determine the ABO blood types, the types of 42 of these samples were demonstrated specifically by treatment with the corresponding anti-A, B or H mAb, but the other 12 samples showed no reactivity with these antibodies (Table 1). The secretor status of former group except one (case no.19) and latter group were secretor (Se) and non-secretor (se), respectively, according to the Lewis system types of their blood. Then, these samples were subjected to the indirect ELISA to examine whether the p 84 concentration depends on the secretor status or not, assuming that the exceptional one (case no.19) was from a Se. There was no significant difference ($p > 0.05$) in the absorbance between Se group (0.163 ± 0.014, $n = 42$) and se group (0.156 ± 0.018, $n = 12$). The data suggests that the concentration of p 84 molecule is independence of the secretor status.

In the sandwich ELISA, the properties of the anti-A, B and H mAbs were assessed by measuring the absorbance values of the above 42 semen samples. The anti-A mAb reacted specifically with semen from donors with blood type A or AB after capture of p 84 by the anti-p 84 mAb bound to the ELISA plate wells (0.68 ∼ 0.13), but not with semen from donors with blood type B or O. The anti-B mAb reacted only with semen from donors with blood type B or AB (1.02 ∼ 0.10), whereas the anti-H mAb reacted with semen from donors/blood types. The mean H activities of semen from donors with blood types A and B were 0.07 ± 0.06 and 0.05

TABLE 1—*The ABO blood typing of semen by sandwich ELISA.*

No.	Absorbance			ABO/p84	ABO/Blood	Lewis/Blood	Se/se
	Anti-A	Anti-B	Anti-H				
1	**0.47***	0.01	0.01	A	A	a−b+	Se
2	**0.21**	0.01	0.05	A	A	a−b+	Se
3	**0.15**	0.01	0.13	A	A	a−b+	Se
4	**0.68**	0.01	0.17	A	A	a−b+	Se
5	**0.40**	0.01	0.16	A	A	a−b+	Se
6	**0.28**	0.01	0.05	A	A	a−b+	Se
7	**0.46**	0.01	0.08	A	A	a−b+	Se
8	**0.37**	0.00	0.02	A	A	a−b+	Se
9	**0.58**	0.00	0.01	A	A	a−b+	Se
10	**0.57**	0.00	0.17	A	A	a−b+	Se
11	**0.24**	0.01	0.05	A	A	a−b+	Se
12	**0.15**	0.01	0.05	A	A	a−b+	Se
13	**0.42**	0.01	0.02	A	A	a−b+	Se
14	**0.13**	0.01	0.01	A	A	a−b+	Se
15	**0.19**	0.00	0.04	A	A	a−b+	Se
16	**0.13**	0.00	0.09	A	A	a−b+	Se
17	**0.32**	0.00	0.10	A	A	a−b+	Se
18	**0.46**	0.00	0.08	A	A	a−b+	Se
19	**0.41**	0.01	0.00	A	A	a−b−	Se
20	0.00	**0.43**	0.04	B	B	a−b+	Se
21	0.01	**0.96**	0.03	B	B	a−b+	Se
22	0.01	**1.02**	0.04	B	B	a−b+	Se
23	0.01	**0.88**	0.05	B	B	a−b+	Se
24	0.01	**0.31**	0.10	B	B	a−b+	Se
25	0.01	0.01	**0.07**	O	O	a−b+	Se
26	0.00	0.02	**0.12**	O	O	a−b+	Se
27	0.00	0.01	**0.21**	O	O	a−b+	Se
28	0.02	0.02	**0.14**	O	O	a−b+	Se
29	0.01	0.01	**0.26**	O	O	a−b+	Se
30	0.00	0.01	**0.08**	O	O	a−b+	Se
31	0.01	0.01	**0.05**	O	O	a−b+	Se
32	0.02	0.02	**0.22**	O	O	a−b+	Se
33	0.01	0.01	**0.13**	O	O	a−b+	Se
34	0.00	0.00	**0.20**	O	O	a−b+	Se
35	0.02	0.01	**0.18**	O	O	a−b+	Se
36	0.01	0.01	**0.23**	O	O	a−b+	Se
37	0.01	0.01	**0.07**	O	O	a−b+	Se
38	**0.37**	**0.85**	0.02	AB	AB	a−b+	Se
39	**0.22**	**0.68**	0.02	AB	AB	a−b+	Se
40	**0.32**	**0.55**	0.03	AB	AB	a−b+	Se
41	**0.16**	**0.10**	0.06	AB	AB	a−b+	Se
42	**0.29**	**0.80**	0.01	AB	AB	a−b+	Se
43	0.01	0.00	0.01	N.D.†	A	a+b−	se
44	0.01	0.00	0.00	N.D.	A	a+b−	se
45	0.01	0.00	0.01	N.D.	A	a+b−	se
46	0.01	0.01	0.02	N.D.	B	a+b−	se
47	0.00	0.00	0.01	N.D.	B	a+b−	se
48	0.00	0.02	0.01	N.D.	B	a+b−	se
49	0.00	0.00	0.01	N.D.	O	a+b−	se
50	0.00	0.01	0.01	N.D.	O	a+b−	se
51	0.00	0.00	0.01	N.D.	O	a+b−	se
52	0.00	0.01	0.01	N.D.	AB	a+b−	se
53	0.02	0.01	0.01	N.D.	AB	a+b−	se
54	0.01	0.01	0.01	N.D.	AB	a+b−	se

* The bold value presented are the positive reaction (absorbance value of over 0.05).
† N.D. indicate not determiable.

± 0.02 for semen from the majority of donors irrespective of blood type. These values however were lower than the minimum absorbance measured with anti-A for semen from donors with blood type A (0.13) and than that by anti-B for those with blood type B (0.31). Therefore, this assay system demonstrated correctly the ABO blood types of semen from secretor individuals.

Specificity of the anti-p 84 mAb for Semen

The anti-p 84 mAb produced by immunizing a mouse with p 84, reacted strongly with human seminal plasma during initial screening. In order to determine whether this antibody is suitable for analysis of forensic samples, saliva, urine, blood, breast milk, and semen were mixed in each body fluid, and subjected to the indirect or sand-

wich ELISA together with vaginal secretions from donors of various blood types to evaluate the specificity of the anti-p 84 mAb (Fig. 1). When serial double-diluted samples of saliva, urine, breast milk, blood, vaginal secretions, and semen were incubated in ELISA plate wells and then subjected to the indirect ELISA, p 84 activity was detected in semen diluted up to 4,000-fold, but no activity was detected in urine, blood or vaginal secretions, even when these samples were not diluted. Saliva and breast milk showed p 84 activity at much lower dilutions, at least 1/400 ~ 1/1,000 less than that of semen, and dried stains of these samples showed no trace of activity. Furthermore, no immunoreactivity corresponding to p 84 was detected in the stains of these two specimens up to 16 times larger than a 0.5 cm by 0.5 cm square (data not shown).

The BGS activities of these specimens were examined by performing the sandwich ELISA following the capture of the BGS molecule (p 84) and evaluated (Fig. 1). The results were acceptable when no A antigenic activity of saliva, urine, breast milk or blood was observed even at no dilution without semen and its stain. Furthermore, vaginal secretions from subjects with blood types A, B, O, and AB showed no antigenic activity.

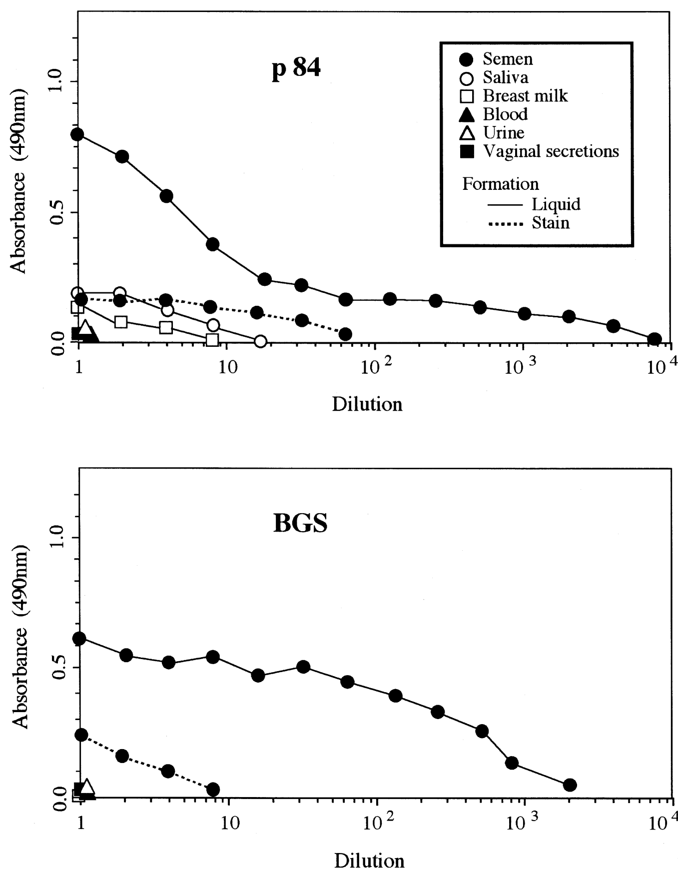

FIG. 1—*Cross reactivity of anti-p 84 mAb in various human body fluids. Antigenic activity of p 84 and BGS in these samples were measured in duplicate by indirect ELISA and sandwich ELISA, respectively described in Materials and Methods. Saliva, urine, blood, semen from 5 individuals (blood type A, Se), breast milk from 2 individuals (blood type A, Se) and vaginal secretions from donors of various blood types were mixed in each body fluid and then double-diluted serially (solid line). A portion of each specimen was blotted on a filter paper, allowed to dry to produce a stain and whose 0.5 by 0.5 cm square was extracted in 330 μL PBS and then double-diluted serially (broken line).*

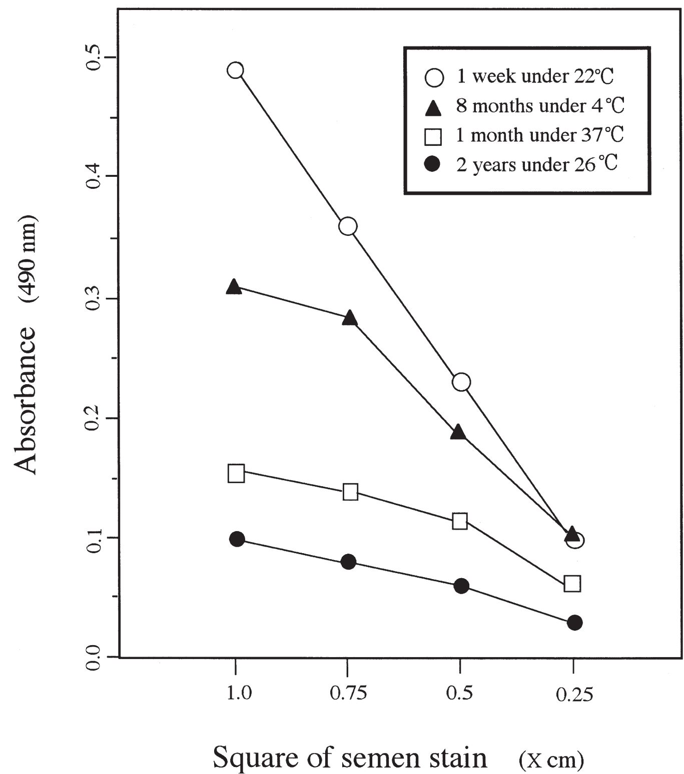

FIG. 2—*Detection of blood group substance (BGS) in semen stains: comparative study of the storage time, storage temperature and stain size. Each square of stained filter paper was extracted with 330 μL PBS and then tested in duplicate.*

Lower Limit of Detection of ABH Antigenic Activity in Seminal Stains

The next step was to establish whether this assay system can be used to determine the ABO blood type in the stains. In order to determine the minimum size of a square of seminal stain stored under various conditions needed for accurate ABO blood typing, semen samples from 5 individuals (blood type A, Se) were mixed and 100-μL aliquots of the mixture were dropped on to filter papers, which were dried and kept at 22, 26, 4, and 37°C for 1 h, 2 years, 8 months, and 1 month, respectively. The stained areas of the filter papers were cut into squares of 4 sizes:1.0 by 1.0, 0.75 by 0.75, 0.5 by 0.5, and 0.25 by 0.25 cm, extracted with 330 μL PBS and then subjected to the sandwich ELISA to detect the BGS activity of semen (Fig. 2). After 1 h at 22°C, the A antigenic activity of the stain decreased, but the activity was always over 0.1, even with the smallest square (0.25 by 0.25 cm). When the A antigenic activity of a 1.0 by 1.0 cm square stain left for 1 h at 22°C was taken as 100%, the activities of the other stains of the same size kept at 4°C for 8 months, 37°C for 1 month and 26°C for 2 years were 63, 31, and 20% of the original activity, respectively. Furthermore, although A antigenic activity could be detected in 0.25 by 0.25 cm square stains kept at 4°C for 8 months and at 37°C for 1 month, no activity was detected in such stains kept at 26°C for 2 years (absorbance < 0.05). These results suggest that the older a dried semen stain becomes the more difficult it is to recover seminal proteins including BGS from it.

Detection of ABH Antigenic Activity in Semen Contaminated with Other Body Fluids

The data presented above demonstrate that our sandwich ELISA can be used for ABO blood typing of semen and seminal stains. The final goal of our study was to establish whether this assay system can detect seminal ABH antigens of semen contaminated by other body fluids. We designed the following experiment to test the semen contaminated by saliva or bloods, at varying volumetric ratios. Semen (blood type B, Se), saliva (blood type A, Se) and whole blood (type A) from 5 individuals were mixed in each body fluid and 100-μL aliquots of this semen mixture were re-mixed with saliva mixture or blood mixture at volumetric ratios of 1: 1, 1:2, 1:4, 1:8, or 1:16. Then, 100-μL aliquots of each mixture were dropped on to filter papers, and the dried stains were cut into 0.5 by 0.5 cm squares, extracted with 330 μL PBS and subjected to the sandwich ELISA. The B antigenic activity of the extract from a 0.5 by 0.5 cm square of only semen stain (its absorbance at 490 nm was 0.294, duplicate assay) was taken as 100% and compared with that of each mixed stain extract. As shown in Fig. 3, when semen was contaminated with saliva or blood at a 1:1 ratio, the BGS activity of the seminal stain was the half of the activity. However, the larger the proportion of saliva or blood, the greater the reduction of the original BGS activity (B), which disappeared when an 8-fold excess of saliva or blood was present. It is of interest that no A antigenic activity of saliva or blood at any mixture ratio was detected in this assay (data not shown).

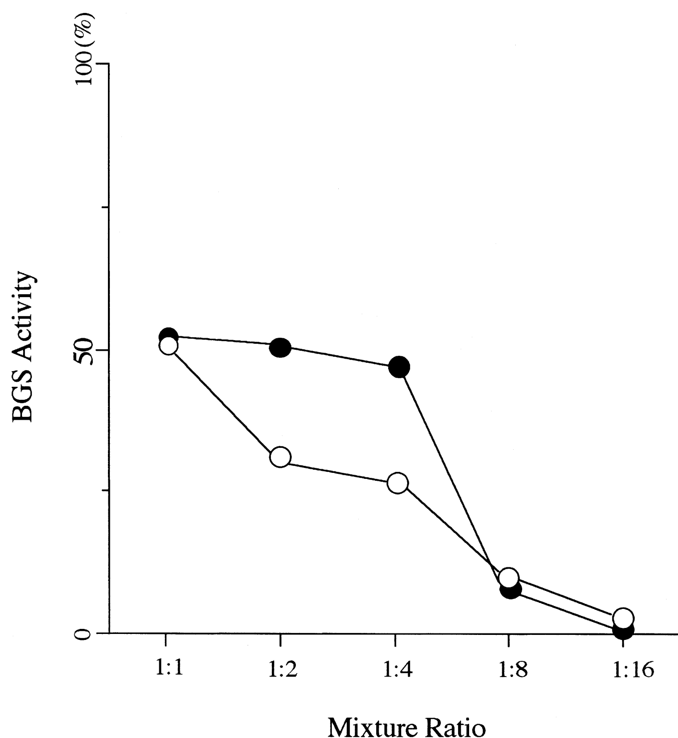

FIG. 3—*Effects of saliva and blood on the determination, by the sandwich ELISA, of the ABO blood type of semen in mixed stains. The open and solid symbols indicate respective blood (A) and saliva (blood type, A, Se), samples, to which semen (blood type, B, Se) was added to produce following semen: other fluid volumetric ratios, 1:1, 1:2, 1:4, 1:8, and 1:16. Each mixture was dropped on to a filter paper, which was air-dried and the stained paper was cut into 0.5 by 0.5 cm squares, each of which was extracted with 330 μL PBS and then the B antigenic activity was determined in duplicate by the sandwich ELISA.*

Application of Sandwich ELISA to the Detection of Seminal BGS in Forensic Casework Samples

The results of forensic casework samples subjected to ABO blood typing of semen by the sandwich ELISA are shown in Table 2. In 8 of 12 casework samples, the ABO blood types of the suspects arrested for these assaults had been determined. The seminal ABO blood types of 7 of them determined by the sandwich ELISA agreed with the ABO blood types of the suspects. Although p 84 was present in a sample from case no. 1, the sandwich ELISA result showed no positive reactions of the anti-A, B or H mAbs. Therefore, this semen was thought to be from a se.

In 2 samples (case nos. 6 and 7), it was not possible to determine the ABO blood type by the absorption-elution test, because the fibers of these specimens caused negative reactions for body fluids, including semen, saliva, blood, sweat, and urine, due to their nonspecific binding to the polyclonal anti-A or B antiserum or *Ulex europaeus* lectin I. The ABO blood type of both suspects involved was AB. However, extracts of these two forensic samples showing

TABLE 2—*Case work study.*

Case Work No.	Blood Type of Persons		Sample	Confirmatory Test for Semen				ABO Blood Typing	
	Victim	Suspect		Acid Phosphatase	p 30	p 84	Sperm	Adsorption-Elution Test	Sandwich ELISA
1	B(Se)	O(se)	bed sheet	++	++	++	(−)	B	se
2	B(Se)	B(Se)	vaginal swab	weak	+	+	+	B	B
3	A(Se)	B(Se)	oral liquid	++	++	++	++	AB	B
4	O(Se)	AB(Se)	tissue paper	++	++	++	++	AB	AB
5	O(Se)	O(Se)	skirt	++	++	+	+	O	O
6	A(Se)	unknown	sweat suit	weak	+	+	(−)	undetectable	A
7	O(Se)	unknown	panties	+	+	+	+	undetectable	B
8	A(Se)	unknown	panties	weak	(−)	(−)	(−)	A	(−)
9	ditto	ditto	panties	+	++	+	+	A	A
10	O(Se)	B(Se)	stockings	++	++	++	+	B	B
11	AB(Se)	O(Se)	vaginal swab	++	++	++	++	AB	O
12	A(Se)	unknown	panties	+	+	+	+	A	A

NOTE: ++, +, weak and (−) in the test of acid phosphatase, p 30 and p 84 indicate strongly positive reaction, positive reaction, weaker than expected and negative reaction, respectively.

++, +, and (−) in sperm search indicate many numbers of sperm in 1 field, 1 sperm or more in several fields and no observation in any field.

positive reactions for semen did contain BGS, detected by the sandwich ELISA.

Although a sample from case no.8 was shown to be blood type A by the absorption-elution test, the sandwich ELISA showed no BGS activity, which agreed with the results of the confirmatory test for semen. BGS was detected in semen samples from four cases nos. 6, 7, 9, and 12, by the sandwich ELISA, but we have not been able to confirm whether these results agree with the ABO blood types of the suspects, because they have not yet been arrested.

Discussion

In a previous study, Sato et al. (1995) demonstrated that the ABO blood type of semen could be determined by identifying a protein with molecular mass of 84 kDa using an electrophoretic method (7). Although this procedure enables a seminal BGS with a molecular mass of 84 kDa to be identified visually, intricate procedures involving immunoblotting with anti-A, B and H mAbs followed by immunoprecipitation are required, making it unsuitable for routine use in the forensic laboratory. The sandwich ELISA for detecting the ABO blood type of semen we performed in this study is simple and much easier to perform than the electrophoretic procedure.

We observed that p 84 was present not only in semen samples from secretors (Se), but also in those from non-secretor (se). However, BGS from p 84 could not be detected in semen from the latter, which suggests strongly that seminal p 84 from non-secretors does not bear ABH sugar epitopes. Therefore, the content of p 84 in semen should be examined by an indirect ELISA using the anti-p 84 mAb before ABO blood typing of semen by the sandwich ELISA.

When the mean A and B antigenic activities of semen from donors with blood type AB (Se) were compared, the former was only 0.33 times that of the latter (Table 1). If serial two-fold diluted semen samples from donors with blood type AB are subjected to the sandwich ELISA, the A antigenic activity will be lost sooner than the B antigenic activity. However, the difference between the A and B antigenic activities in semen from donors with blood type AB is thought to be attributable to the different titers of the mAbs, rather than different amounts of the sugar epitopes. The mean A antigenic activity in semen from donors with blood type A was about 0.47 times that of the B antigen in semen from donors with blood type B, the same relative activities as semen from donors with blood type AB. Therefore, the titers of the anti-A and B mAbs should be adjusted to the same relative value as that of donors with blood type AB (Se) before the ABO blood type of semen is determined by the sandwich ELISA.

Our experiments using the sandwich ELISA method showed that: 1) The anti-p 84 mAb we used is highly specific, 2) The antigenic activity of p 84 is stable, and 3) p 84 can be used as a marker for ABO blood typing of semen, semen stains and the stains of semen contaminated with blood or saliva. Our results demonstrated that this assay system can detect BGS of p 84 in a seminal stain with a size of 0.5 by 0.5 cm square or greater, even after storage for a long time under various environmental conditions (Fig. 2). Moreover, we showed that this assay system can detect the BGS of p 84 in a stain composed of semen and saliva or blood (Fig. 3). Although the BGS activity of semen was about 90% of its original value when contaminated with a 4-fold excess of saliva, its activity fell to about 50% in the presence of a 4-fold excess of blood. This difference is probably due to the different protein concentrations of saliva and blood. The finding of our previous study that the BGS activity of semen was not affected by adding vaginal secretions up to a 10-fold excess supports this suggestion (7). These results show that the lower the protein concentration of a body fluid added to semen, the higher the relative seminal protein concentration of the extract will be. The major advantage of the ELISA method we used in this study is that just the BGS of semen can be captured even if the semen sample is contaminated with other body fluids.

Finally, we examined whether seminal BGS could be detected in evidential samples obtained from several sexual assault victims by carrying out the sandwich ELISA and whether this method can be applied to the analysis of forensic materials. Our results suggest strongly that this method can be used to determine the ABO blood type of semen after removal of the victim's BGS (Table 2).

We believe and hope that this sandwich ELISA can be used widely in many forensic laboratories for determining the ABO blood type of semen which is contaminated with vaginal secretions, saliva or blood occurs frequently in cases of sexual assault. Therefore, we will supply the anti-p 84 mAb to researchers who wish to use it and we are now studying the molecular properties of p 84 by analyzing its DNA sequence.

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