ELSEVIER

Forensic Science International
91 (1998) 19–28

Forensic Science International

Detection of human seminal γ-glutamyl transpeptidase in stains using sandwich ELISA

Sumiko Abe[a,*], Satoshi Kunii[a], Teizo Fujita[b], Kouichi Hiraiwa[a]

[a]*Department of Legal Medicine, Fukushima Medical College, 1 Hikarigaoka, Fukushima, 960-12, Japan*
[b]*Department of Biochemistry, Fukushima Medical College, 1 Hikarigaoka, Fukushima, 960-12, Japan*

Received 11 February 1997; received in revised form 8 October 1997; accepted 9 October 1997

Abstract

A sensitive and specific sandwich ELISA for human seminal γ-glutamyl transpeptidase (γ-GTP) was developed using a combination of monoclonal antibodies, SG1 and SG3, which we produced. For semen identification in forensic samples, we modified the assay so as to be more sensitive and to establish efficient extracting conditions. After testing the extracting abilities of several detergents, CHAPS and deoxy-BIGCHAP were chosen as the solubilizer. Polystyrene beads coated with SG1 were incubated with samples extracted by the detergents, and further with biotinylated SG3, followed by peroxidase-labeled streptavidin. γ-GTP was detected only in seminal samples. The sensitivity of this assay was 0.01 ng/ml of seminal γ-GTP equivalent to 10^7 times diluted semen, which was ten times as compared with the previous plate assay. No significant seminal γ-GTP was detected in other biological stains such as blood, saliva and vaginal smear. The extract of a 500 fold diluted seminal stain, 8 months old, showed the detection limit. Seminal γ-GTP was detectable even in 14-year-old stains. © 1998 Elsevier Science Ireland Ltd.

Keywords: Semen specific γ-GTP; Monoclonal antibodies; Sandwich ELISA; Seminal stains; Detergents; Semen identification

1. Introduction

In forensic case work, the presence of semen can be proven by the microscopic detection of spermatozoa or by the presence of semen-specific components such as prostate specific antigen (PSA) [1], prostatic acid phosphatase (PAP) [2], γ-seminopro-

*Corresponding author. Tel.: +81 245-48-2111; fax: +81 245-49-3874; e-mail: sumiko@cc.fmu.ac.jp

PII S0379-0738(97)00169-2

tein [3], lactate dehydrogenase isoenzyme (LDH-X) [4] and so forth [5,6]. These are now accepted as markers for detecting semen even in cases including vasectomized or azospermic males. Such markers are detected by crossover or rocket electrophoresis, enzyme activity assay or immunoprecipitation, but sensitivities of these methods are still discussed. Enzyme-linked immunosorbent assay (ELISA) is known to be several times more sensitive than the former methods [7–9].

γ-Glutamyl transpeptidase (γ-GTP, EC.2.3.2.2.) catalyzes the transfer of the γ-glutamyl moiety of glutathione and certain other γ-glutamyl peptides to amino acids or to peptides, and shows high activity in human semen. This enzyme, which is primarily a membrane-bound glycoprotein, has been found in a wide variety of human tissues; kidney, liver, pancreas, jejunum, seminal vesicle and so on, of which the kidney proximal tubules exhibits by far the highest activity [10]. We previously reported that seminal γ-GTP is semen specific and different from renal or hepatic enzyme with respect to amino acid composition, molecular masses of two subunits and immunological characters [11]. Then we produced monoclonal anti-seminal γ-GTP antibodies, SG1 and SG3, which react only with male reproductive organs and have one's own epitope [12].

Immunohistochemical staining with SG1 was positive in the epithelial cells of the ductus epididymidis, seminal vesicle and prostate gland, whereas no reaction was noted in the brush border of proximal convoluted tubules in the kidney. SG3 reacted only with the epithelial cells of seminal vesicle. Using SG1 and SG3, a sensitive and specific sandwich ELISA for human seminal γ-GTP was created [13]. The detection limit of this assay was estimated to be 1 ng/ml of seminal γ-GTP equivalent to approximately 10^5 times diluted semen. This ELISA was specific for seminal γ-GTP without cross-reactivity of renal or hepatic γ-GTP, normal blood serum, non-coital vaginal fluid, saliva or sweat. For the identification of semen in forensic samples, this assay has been improved by using polystyrene beads instead of microtiter plate, and a sensitivity of less than 0.01 ng/ml seminal γ-GTP is routinely achieved. This paper also presents efficient extracting conditions for seminal γ-GTP from forensic samples.

2. Materials and methods

2.1. Sample preparation

Pooled semen obtained from healthy male volunteers was centrifuged for 30 min at $2100\times g$, and the supernatant was stored at −80°C until use. Seminal stains were made by spreading 500 μl of semen on filter paper (No. 3, Whatman, Kent, England) and kept at room temperature. Some of the seminal stains were exposed to UV (8-cm distance at a wave length of 253.7 and 365.0 nm lights) or high temperature (in an oven at 37 or 70°C) to test their influence on antigenicity of seminal γ-GTP. Semen-free vaginal swabs were obtained from the patients by courtesy of Dr. K. Yanagida, Department of Obstetrics and Gynecology, Fukushima Medical College, and semen contamination was examined under a light microscope. A part of the semen was incubated with blood, saliva or vaginal fluid from female donors at 37°C for 2 h and then spread on a filter paper.

Pairs of stains were made on filter paper of nineteen different kinds of household products such as beverages, seasonings, lotions and cleaning agents. Each product was mixed with a 1/10 volume of semen or distilled water. All the stains were allowed to dry for a month under laboratory conditions.

Stains (filter paper) and swabs (cotton) from forensic materials were examined for PAP and sperm immediately after they were obtained and were dried and kept at room temperature. γ-GTP ELISA was run several years later, because we have only a few cases a year. A 6-year-old sample was the oldest.

2.2. Extraction

For extraction of seminal γ-GTP from the stains, we tested the following five kinds of detergents; 3[(3-cholamidopropyl) dimethylammonio]propanesulfonic acid(CHAPS), 3-[(3-cholamidopropyl) dimethylammonio]-2-hydroxypropanesulfonic acid(CHAPSO), *N*,*N*-bis(3-D-gluconamido-propyl)cholamide (BIGCHAP), *N*,*N*-bis(3-D-gluconamido-propyl)deoxycholamide(deoxyl-BIGCHAP) and *n*-octyl-β-D-glucopyranoside (OG) (Dojindo, Kumamoto, Japan). Their critical micelle concentrations(CMC) are 8 mM (CHAPS and CHAPSO), 2.9 mM (BIGCHAP), 1.4 mM (deoxy-BIGCHAP) and 9 mM (OG), respectively.

Stains were cut into pieces 3×3 mm in size. 450 μl of detergent or distilled water was added to three of them. They were agitated overnight (60 rpm) at room temperature. After centrifugation at 12 000 rpm for 5 min, 25 μl of 1:25 diluted supernatant with PBS was assayed by ELISA. In the case of vaginal swabs, they were weighed, then extracted with detergents (10 mg cotton/ml detergent). PAP activity of the supernatant of extract was simultaneously measured by the method of Ostrowski et al. [14].

2.3. Monoclonal antibodies

Monoclonal antibodies against human seminal γ-GTP, SG1 and SG3, were prepared as previously described [12]. SG3 was biotinylated with Biotin-$(AC_5)_2$-Sulfo-Osu (Dojindo Lab., Kumamoto, Japan).

2.4. ELISA procedure

The previous ELISA method was slightly modified as follows [13]. Polystyrene beads (3.18 cm, Wako, Osaka, Japan) were coated with SG1 (4.5 μg/ml), next soaked in 1 mg/ml BSA. The bead was incubated with antigen in a total volume of 0.15 ml at room temperature for 2 h. After 3 washes with PBS containing 0.05% Tween, the bead was incubated with biotinylated SG3 (1.7 μg/ml), followed by peroxidase-labeled streptavidin (Dako, Carpinteria, USA). Finally, the substrate solution was added. After incubation for 0.5 h, the color reaction was terminated by adding 50 μl of 0.5% sodium azide, and the optical density was measured at 405 nm. Blank and several points of diluted semen (10^7, 10^6, 10^5) were run in each assay for calibration curve. All samples were tested in duplicate at least.

3. Results

3.1. Reproducibility

We created a new calibration curve because the method was modified. Each assay was done with blank (O.D. 0.196±0.019) and standards. For the standard γ-GTP concentration (10^5 diluted semen corresponding to 1 ng/ml of γ-GTP), the intra-assay coefficient of variation (C.V.%) was below 10% (1.259±0.019), and the interassay C.V.% was below 20% (1.287±0.246).

3.2. Extraction

To get the efficient extract condition, we tested five detergents at 2 fold CMC first. 1-month-old seminal stains were used for extraction tests. We also checked the influence of the detergents on the antigenicity of seminal γ-GTP by incubation of semen and detergent mixtures for 2 h at room temperature. Table 1 shows the extract abilities and the influence of the detergents for detecting γ-GTP by ELISA. Compared with the distilled water, CHAPSO and BIGCHAP interfered with antigen detection in cases of semen directly diluted with them ($1:10^5$). CHAPS, deoxy-BIGCHAP and OG enhanced the sensitivities at an O.D. value of 35% more than those of distilled water. The extract abilities of these three were also better than water. From these results, CHAPSO and BIGCHAP were excluded from the further experiments.

Table 2 shows the extracting abilities of three detergents at various concentrations. Optimum concentrations for the extraction of seminal γ-GTP were obtained with 2×CMC CHAPS (16 mM), 1.5×CMC deoxy-BIGCHAP (2.1 mM) and 1.5×CMC OG (13.5 mM), respectively. OG gave lower ability than CHAPS and deoxy-BIGCHAP. CHAPS and deoxy-BIGCHAP would be satisfactory detergents for solubilization of γ-GTP from seminal stains.

We have reported that semen contains approximate 100 μg/ml of γ-GTP [11]. As the stains on filter paper were prepared in rectangular size of 50×60 mm with 500 μl of semen, it is expected that 40 ng/ml of γ-GTP may be detectable. Seminal γ-GTP extractions from 1-month-old stains with the above two detergents resulted in the following recoveries; 2.8 ng (7%) with 16 mM CHAPS, and 3.2 ng (8%) with 2.1 mM

Table 1
Sensitivity of seminal γ-GTP in various detergents

	O.D. 405 nm	
Detergent	10^5 Diluted semen	Seminal stains
None (H_2O)	0.533±0.008	0.478±0.003
16 mM CHAPS	0.732±0.033	0.709±0.067
16 mM CHAPSO	0.261±0.014	0.275±0.028
5.8 mM BIGCHAP	0.214±0.008	0.477±0.028
2.8 mM deoxy-BIGCHAP	0.746±0.114	0.893±0.165
18 mM OTG	0.642±0.021	0.685±0.129

Values are means±S.D. in 3 separate experiments.

Table 2
Effect of detergent concentration on extraction from stains

Detergent concentration	CHAPS	Deoxy-BIGCHAP	OG
None(H_2O)	0.252±0.056	0.254±0.013	0.258±0.027
×0.5 CMC	0.492±0.116	0.420±0.044	0.275±0.017
×1.0 CMC	0.691±0.058	0.584±0.027	0.322±0.017
×1.5 CMC	0.851±0.136	1.649±0.132	0.435±0.078
×2.0 CMC	1.141±0.136	1.548±0.007	0.324±0.043
×4.0 CMC	0.892±0.085	1.023±0.037	0.258±0.027

deoxy-BIGCHAP. Protein assay was done in the same extractions. Original semen contained 68 mg/ml protein, thus the extract from three pieces of the samples might contain 680 μg/ml of protein. In PBS, CHAPS and deoxy-BIGCHAP extracts, 350, 480 and 420 μg/ml protein were detected. More than 50% of protein was extracted from 1-month-old stains with each detergent.

3.3. Sensitivity and dilution factor

The sensitivity of this assay system proved to be 0.01 ng/ml of seminal γ-GTP equivalent to 10^7 diluted semen (0.109±0.045). We made 2-month-old diluted semen stains (×10, ×20, ×50, ×100, ×200, ×500, ×1000) to check the dilution limit by ELISA. As the detergent, 16 mM CHAPS was used for this experiment. Compared with the expected amount, 6.8% of seminal γ-GTP was detectable in 1:10 diluted stains, 5.2% in 1:20, 6.5% in 1:50. The trace of γ-GTP was detected in 100 times and more diluted stains. No significant γ-GTP was detected in 1000 times diluted stains. The extract of 500 times diluted seminal stains showed the detection limit at O.D. value (O.D. 0.068=0.09 ng of γ-GTP/ml).

3.4. Stability

After the stains were exposed to heat (37°C, 70°C) or UV for several hours, γ-GTP extracted with CHAPS or deoxy-BIGCHAP was measured (Table 3). There was no significant difference between non-treated (stood at room temperature) and 37°C-treated samples. When the stains were exposed to heat at 70°C for 14 h, 40% of antigen was detected in seminal stains. The antigenicity was most affected by UV. 14 h of UV exposure reduced γ-GTP detection to 10%, while almost the same amount of protein was detected in all extracts even after UV exposure.

The stability of antigenicity of seminal γ-GTP in biological fluid was also tested (Table 4). Blood, vaginal fluid, saliva or PBS did not contain endogeneous γ-GTP. Semen was mixed with blood, semen-free vaginal fluid, saliva or PBS and incubated at 37°C for 2 h, then spread onto filter paper. One month later, they were extracted and assayed. Compared with the mixture with PBS, 40% of γ-GTP antigenicity was lost in saliva, 30% with vaginal fluid and 20% with blood, respectively.

Table 3
Influence of heat or UV-rays exposure

Treatment		CHAPS ×2 CMC	Deoxy-BIGCHAP ×1.5 CMC
None treated		1.666±0.247	1.571±0.141
37°C	1 h	1.360±0.159	1.154±0.113
	3 h	1.757±0.157	1.917±0.184
	5 h	1.709±0.180	1.567±0.131
	8 h	1.598±0.120	1.501±0.062
	14 h	1.099±0.087	1.646±0.106
70°C	1 h	1.172±0.075	0.695±0.054
	3 h	1.180±0.120	0.861±0.083
	5 h	1.101±0.262	0.562±0.096
	8 h	0.891±0.132	0.601±0.108
	14 h	0.698±0.091	0.673±0.098
UV	1 h	0.592±0.070	0.376±0.125
	3 h	0.385±0.046	0.270±0.054
	5 h	0.429±0.156	0.218±0.069
	8 h	0.317±0.036	0.118±0.025
	14 h	0.268±0.058	0.103±0.033

Data values are means of 3 separate experiments.

Table 4
Seminal γ-GTP antigenicity mixed in biological fluids

	CHAPS	Deoxy-BIGCHAP
None	0.007±0.006	0.007±0.003
+semen	0.811±0.239	0.669±0.132
Blood	0.013±0.005	0.015±0.005
+semen	0.605±0.087	0.596±0.022
Vaginal fluid	0.006±0.003	0.008±0.004
+semen	0.551±0.101	0.483±0.007
Saliva	0.008±0.006	0.010±0.006
+semen	0.495±0.051	0.410±0.057

Data values are means of 4 separate experiments.

Table 5
Effect of time on seminal γ-GTP detection

	H_2O	16 mM CHAPS	2.1 mM Deoxy-BIGCHAP
Blank	0.003±0.003	0.005±0.004	−0.003±0.004
1 month old	1.503±0.082	2.054±0.107	2.054±0.217
2 months old	0.660±0.063	2.106±0.153	1.634±0.302
4 months old	0.254±0.035	1.971±0.037	1.560±0.331
6 months old	0.207±0.040	1.566±0.004	0.875±0.010

Data values are means of 5 separate experiments.

Table 5 shows the effect of time on seminal γ-GTP detection. In 6-month-old seminal stains, 40 to 50% of γ-GTP was found when compared with 1-month-old samples.

The old seminal stains were analyzed by ELISA. Slight brownish colored semen spot could be identified from blank space on filter paper or cloth. Both colored spot and blank space were extracted and assayed by ELISA without dilution. Old blank areas did not show a false positive reaction. 10-year-old semen stains gave 0.1 to 1.5 at O.D. 405 nm. Seminal γ-GTP was detectable even in 14-year-old stains (0.04–0.25).

The results of γ-GTP testing on the paired stains are shown in Table 6. None of the household contaminants tested gave a false positive result for seminal γ-GTP except cow milk (±, O.D. 0.054±0.003). Several contaminated stains produced false negative results (yogurt, mayonnaise and bleach). With lotion, face cream or shampoo, γ-GTP was found to be weaker than expected.

3.5. Casework samples

The results of casework samples tested for PAP, sperm and γ-GTP are shown in Fig. 1. The oldest sample was 6 years old. However PAP activity was also measured in the same extracts, its activity was no longer detectable. The extracts of these samples were assayed without dilution. A total of 41 samples were categorized into 3 groups: Group 1, sperm negative and PAP negative in 25 cases; Group 2, sperm negative but PAP positive in 13 cases; Group 3, sperm positive and PAP positive in 3 cases. There was no case of PAP negative, but sperm positive. No significant γ-GTP was detected in

Table 6
Paired stain study

Substance	Alone	+Semen
Tea	−	+
Coffee	−	+
Cocoa	−	+
Milk	±	+
Yogurt	−	±
Grapefruit Juice	−	+[a]
Juice	−	+
Japanese sake	−	+
Vinegar	−	+
Soy sauce	−	+
Ketchup	−	+
Mayonnaise	−	±
Lotion	−	+[a]
Face cream	−	+[a]
Shampoo	−	+[a]
Hair conditioner	−	+
Dishwashing liquid	−	+
Bleach	−	−

−: Negative reaction (O.D. <0.050).
+: Positive reaction.
[a] Weaker than expected.

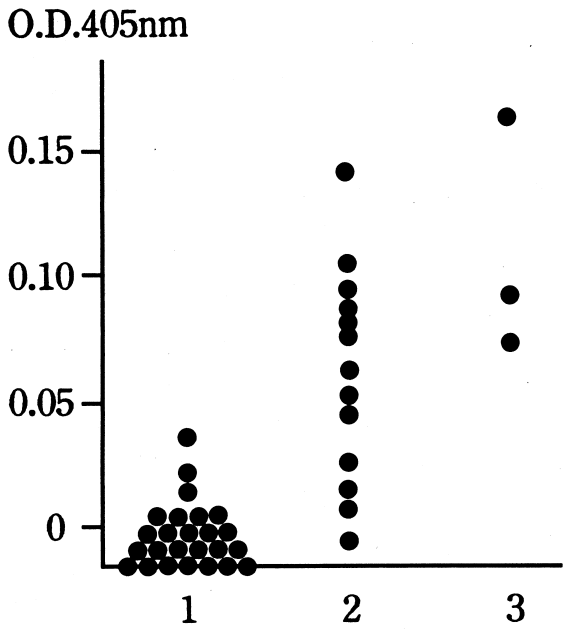

Fig. 1. Seminal γ-GTP level in casework samples compared with sperm search and PAP. Group 1: Sperm(−), PAP(−); Group 2: Sperm(−), PAP(+); Group 3: Sperm(+), PAP(+).

Group 1. In Group 2, 8 samples were γ-GTP positive, while 5 were negative. All of the three samples in Group 3 were γ-GTP positive.

4. Discussion

We reported the development and validity of a sensitive sandwich ELISA, which proved to be a reliable tool for determining seminal γ-GTP level in forensic samples. The sensitivity of this ELISA was below 0.01 ng/ml equivalent 10^7 diluted semen. In prepared stainings, the detection limit was 500 times diluted semen stains.

For solubilization of membrane-associated protein, many detergents were developed [15,16]. These are widely used for solubilization and subsequent isolation of membrane proteins, due to their high CMC. They have been employed for extracting ABO blood group antigens from staining samples in forensic case work [17]. Sagisaka et al. reported that 0.125% of deoxy-BIGCHAP (=1×CMC) could extract ABH antigens from blood stains up to 10 years old [18]. In this method, CHAPSO and BIGCHAP gave adverse effects on the detection of seminal γ-GTP. For solubilization of seminal γ-GTP from biological stains, CHAPS and deoxy-BIGCHAP worked better than other detergents (Table 1). Using CHAPS or deoxy-BIGCHAP, most of the protein (60–70%) was extracted from 1-month-old stains. On the other hand, less than 10% of seminal γ-GTP was detectable in the same extract. It has been proven that two detergents do not

interfere with the detection of seminal γ-GTP by the direct incubation test (Table 1). Therefore, there may be a strong association between γ-GTP sugar moiety or other structures and filter paper fiber as compared with other proteins.

Dilution factor affected detection of seminal γ-GTP. In 14-year-old seminal stains prepared without dilution, γ-GTP could be detected by this assay, but not in highly-diluted stains.

Under mild heat condition, there is no big effect on antigenicity of seminal γ-GTP. UV lights affected the detection of γ-GTP, but did not effect the protein recovery. Thus, UV lights may destroy the epitope structure of γ-GTP (Table 3). However, the condition we tested is so severe that it may not happen in practice, since natural UV light is about one percent of this condition.

Cow milk showed a slight positive (±) reaction. Histochemical and biochemical investigations showed that marked activities of γ-GTP were observed in the cytoplasma of glandular epithelial cells of human breast [19] and in the lactating mammary tissue from cow and rat [20]. Milk contains a secretory type of γ-GTP. This might cause very weak cross-reaction with cow milk. Since yogurt and mayonnaise are oil-rich products, epitopes of seminal γ-GTP may be masked, or immunoreaction may be interfered with. These are the reasons why lotion and face cream might give a weaker positive reaction than expected. In the case of PSA detection by ELISA, shampoo, liquid laundry detergent and dish washer liquid were reported to give the false negatives [21]. It appears that those containing detergents interfered with ELISA. Sodium hypochlorite, a major content of bleach, may destroy the antigen. There is a need to consider domestic contaminant in the casework samples.

Seminal γ-GTP assay was successful in identifying semen in casework samples. Seminal γ-GTP was positive in both PAP and sperm positive samples. No γ-GTP was observed in both PAP and sperm negative samples. Eight samples were found to be positive for γ-GTP and PAP, and negative for sperm. These may be azospermic semen or judged as negative ones due to the limitation of the sperm search procedure. The routine test for seminal γ-GTP by ELISA can be helpful for identifying semen in casework samples without sperm but PAP positive.

Acknowledgements

The authors wish to thank Drs. R. Tsuda and I. Nakasono, Department of Legal Medicine, Nagasaki University School of Medicine, for providing casework samples. This work was partly supported by Fukushima Society for the Promotion of Medicine.

References

[1] G.F. Sensabaugh, Isolation and characterization of a semen-specific protein from human seminal plasma: a potential new marker for semen identification, J. Forensic Sci. 23 (1978) 106–115.

[2] P.F. Hansen, Determination of the ‘acid’ phosphatase as a new method for medico–legal demonstration of sperm spots, Acta Pathol. Microbiol. Scans. 23 (1946) 187–214.

[3] M. Hara, Y. Koyanagi, T. Inoue, T. Fukuyama, Some physicochemical characteristics of 'γ-seminoprotein', an antigenic component specific for human seminal plasma, Jpn. J. Legal Med. 25 (1971) 322–324.
[4] R.H. Mokashi, M.S. Madiwale, The use of sperm-specific lactate dehydrogenase isozyme for the identification of semen in dried stains, Forensic Sci. 8 (1976) 269–275.
[5] K. Caldwell, E.T. Blake, G.F. Sensabaugh, Sperm diaphorase: genetic polymorphism of a sperm-specific enzyme in man, Science 191 (1976) 1185–1187.
[6] M.E. Lawton, J.G. Sutton, A study of leucine aminopeptidase as a possible means of identifying semen, J. Forensic Sci. 22 (1982) 193–198.
[7] L.I. Stowell, L.E. Sharman, K. Hamel, An enzyme-linked immunosorbent assay (ELISA) for prostate-specific antigen, Forensic Sci. Int. 50 (1991) 125–138.
[8] R. Tsuda, M. Hara, K. Sagawa, Demonstration of seminal stains by sandwich ELISA using monoclonal γ-seminoprotein antibody bound to acrylbutadienestyrene beads, Jpn. J. Legal Med. 38 (1984) 83–87.
[9] A. Tsutsumi, Y. Yamamoto, K. Okamura et al, Identification of human semen by a dot-ELISA method for seminal vesicle specific antigen (SVSA), Act. Crim. Japon 55 (1989) 204–212.
[10] S.S. Tate, A. Meister, γ-Glutamyl transpeptidase from kidney, Methods Enzymol. 113 (1985) 400–419.
[11] S. Abe, H. Kochi, K. Hiraiwa, Purification and immunological characterization of a new form of γ-glutamyltransferase of human semen, Biochim. Biophys. Acta 1077 (1991) 259–264.
[12] S. Abe, H. Gunji, T. Fujita, K. Hiraiwa, Immunocytochemical study of seminal γ-glutamyltransferase using monoclonal antibodies, Cell Tissue Res. 277 (1994) 33–38.
[13] S. Abe, H. Gunji, T. Fujita, K. Hiraiwa, A sandwich enzyme-linked immunosorbent assay for human seminal γ-glutamyl transpeptidase, Forensic Sci. Int. 76 (1995) 35–45.
[14] W. Ostrowski, E.A. Barnard, Acid phosphomonoesterase of human prostate. Phosphate transfer reactions and comparison with alkaline phosphatase, Biochim. Biophys. Acta 250 (1971) 131–142.
[15] L.M. Hjelmeland, A nondenaturing zwitterionic detergent for membrane biochemistry: design and synthesis, Proc. Natl. Acad. Sci. USA 77 (1980) 6368–6370.
[16] C. Baron, T.E. Thompson, Solubilization of bacterial membrane protein using alkyl glucosides and dioctanoyl phosphatidylcholine, Biochim. Biophys. Acta 382 (1975) 276–285.
[17] J.P. McCabe, S.M. Fletcher, M.N. Jones, The effects of detergent on the enzyme-linked immunosorbent assay (ELISA) of blood group substances, J. Immunol. Methods 108 (1988) 129–135.
[18] K. Sagisaka, T. Yokoi, I.M. Sebetan, The use of deoxy-BIGCHAP to extract ABH antigens from blood stains for blood grouping by ELISA, Act. Crim. Japon 55 (1989) 8–12.
[19] F. Binkley, M.L. Wiesemann, D.P. Groth, R.W. Powell, γ-Glutamyltransferase: a secretory enzyme, FEBS Lett. 51 (1975) 168–170.
[20] S.S. Tate, Enzymes of mercapturic acid formation, in: W.J. Jakoby (Ed.), Enzymatic Basis of Detoxication, vol. 2, Academic Press, New York, 1980, pp. 95–120.
[21] E.D. Johnson, T.M. Kotowski, Detection of prostate specific antigen by ELISA, J. Forensic Sci. 38 (1993) 250–258.