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γ-Glutamyltransferase Test as a New Tool for Identification of Seminal Stains

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Summary. A simple qualitative method for identification of seminal stains based on a high activity of γ-glutamyltransferase (γ-GTP) in human semen is described. It employs the release of α-naphthylamine from N-γ-glutamyl-α-naphthylamide by the γ-GTP action; α-naphthylamine couples with Fast Garnet GBC salt to produce a strong brownish-red color. The data on its simplicity, specificity, and stability show that the present method is suitable for medicolegal examination of seminal stains as a preliminary test.

Key words: γ-Glutamyltransferase, identification of semen – Seminal stains, identification by γ-glutamyltransferase

Zusammenfassung. Es wird eine einfache, qualitative Methode zur Identifizierung von Spermaspuren beschrieben, die auf der hohen γ-Glutamyltransferase-Aktivität (γ-GTP) des menschlichen Sperma basiert. Dabei wird die Abspaltung von α-Naphthylamin von N-γ-Glutamyl-α-naphthylamid durch das Enzym benutzt, wobei α-Naphthylamin mit Fast Garnet GBC-Salz gekoppelt eine tief braunrote Farbe bildet. Die Ergebnisse der Untersuchung bestätigen die Einfachheit, Spezifität und Stabilität dieser Methode als Vortest zur forensischen Untersuchung von Spermaspuren.

Schlüsselwörter: γ-Glutamyltransferase-Aktivität (γ-GTP) – Sperma, Identifizierung durch γ-GTP

Rosalki and Rowe [1] discovered that human seminal fluid contains an extremely high activity of γ-glutamyltransferase (γ-GTP, formerly γ-glutamyl transpeptidase, EC 2.3.2.2) and suggested that γ-GTP determination could be of value in a forensic work for detection of suspected seminal fluid stains. Nakanishi [2, 3] studied, by a quantitative analysis, the usefulness of γ-GTP for identification of semen from a medicolegal aspect, and claimed that γ-GTP is advantageous in that this enzyme is much more stable than acid phosphatase (ACP) which is commonly used to identify semen as a preliminary test.

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Since quantiative methods are considered unsuitable for actual forensic examinations, in this paper, we present details of a simple qualitative method for demonstration of γ-GTP activity as a new tool for identification of human semen. It is based on a histochemical technique of Albert et al. [4].

Material and Methods

Chemicals

N-γ-Glutamyl-α-naphthylamide, glycylglycine-HCl, and Fast Garnet GBC salt were purchased from Sigma Chemical Company, St. Louis, MO, USA. Other common chemicals used were of the highest purity commercially available.

Stains

Human semen was collected from volunteers by masturbation. Various human body fluids including semen were dropped or smeared on filter paper (Toyo Roshi No. 2, Tokyo). The paper was allowed to dry at room temperature for a few hours and was then cut into small pieces (2 by 5 mm). Seminal stains kept at room temperature for periods of 1 week to 23 years in our laboratory were also used.

Reagents

Reagent I. Twenty milligrams of N-γ-glutamyl-α-naphthylamide and 100 mg of glycylglycine-HCl were dissolved in 5 ml of distilled water by heating at 100° C, and after cooling the pH was adjusted to 7.2–7.8 by dropping 1 N NaOH solution.

Reagent II. Five milligrams of Fast Garnet GBC salt was dissolved in 5 ml of distilled water. Both reagents were stable more than 2 weeks in a refrigerator.

Procedure

Various conditions of the assay were tested and the following procedure is recommended as a standard assay. A small piece of the stained material was placed at the bottom of a small test tube. One drop of Reagent I and one drop of Reagent II were added to the stain, which was then incubated at 37° C for 5–10 min in a water bath. As a blank test, unstained filter paper of the same size was subjected to the assay. After incubation, a strong brownish-red color developed which was observed by the naked eye.

Results

To confirm that the color reaction is due to γ-GTP activity present in seminal stains, we heated them at 100° C for 30 min in a dry heat box, prior to the assay. This resulted in a remarkable weakening of the color developed. Furthermore, the addition of cupric sulfate (1 mM), a known inhibitor of γ-GTP [5], to the reaction mixture suppressed the reaction almost completely, showing that the reaction is catalyzed by γ-GTP.

To check the sensitivity of the present method, five human semen samples were diluted stepwise, dropped on filter paper, and dried to make small stains, which were then treated by the standard procedure. As a result, all samples were positive at least in a fourfold dilution.

Table 1. γ-GTP test on human seminal stains of various ages

Age of stain	Tested, *n*	Positive, *n*	Negative, *n*
1 day to 1 month	19	19	0
2–6 months	12	12	0
7 months to 1 year	10	10	0
2 years	8	8	0
3–5 years	6	6	0
6–10 years	4	4	0
11–23 years	11	10	1

Table 2. γ-GTP test on the stains of various human body fluids

Human body fluid	Tested, *n*	Positive, *n*	Negative, *n*
Semen	49	49	0
Blood	20	0	20
Serum	20	0	20
Saliva	11	0	11
Nasal discharge	7	0	7
Tears	4	0	4
Breast milk	9	7	2
Sweat	8	0	8
Vaginal fluid	15	3	12
Urine	20	0	20
Feces	4	0	4

Seminal stains left at room temperature for various periods were subjected to the present method. As shown in Table 1, all the samples were positive up to 10 years. Only one of 11 samples was negative after 11–23 years of aging.

The results on various human body fluids are presented in Table 2. Except seminal stains, a fairly strong color was observed for seven samples of nine breast milk stains. Although three of 15 vaginal fluid samples were positive, the color was much less intense than that of seminal stains. All other body fluids gave negative results.

The present reaction was checked for the stains of various common vegetables and fruits as listed in Tables 3 and 4. Although a majority of the samples was negative, some stains, such as green pea, broad bean, onion, strawberry, apple and plum, gave weak positive results. It should be noted that cauliflower, which is strongly positive in the ACP test [6], was negative.

Table 3. γ-GTP test on the stains of vegetable extracts

Vegetable	Tested, *n*	Positive, *n*	Negative, *n*
Cabbage	2	0	2
Carrot	2	0	2
Cucumber	2	0	2
Lettuce	2	0	2
Radish	2	0	2
White potato	2	0	2
Barley malt	2	0	2
Cauliflower	3	0	3
Broccoli	2	0	2
Green pepper	2	0	2
Spinach	2	0	2
Kidney bean	3	0	3
Green pea	3	1	2
Broad bean	2	2	0
Onion	2	1	1

Table 4. γ-GTP test on the stains of fruit extracts

Fruit	Tested, *n*	Positive, *n*	Negative, *n*
Grape	2	0	2
Grapefruit	3	0	3
Loquat	3	0	3
Melon	3	0	3
Mandarin orange	3	0	3
Watermelon	3	0	3
Banana	2	0	2
Mango	4	0	4
Pineapple	3	0	3
Papaya	1	0	1
Tomato	2	0	2
Kiwi fruit	1	0	1
Strawberry	2	2	0
Apple	2	1	1
Plum	2	1	1

Discussion

ACP test has been widely used for medicolegal identification of seminal stains as a preliminary test [6]. In this paper, we have presented a different enzymatic method; it employs γ-GTP activity as a marker of human semen. The key reaction is the release of α-naphthylamine from N-γ-glutamyl-α-naphthylamide by the GTP action [4]: α-naphthylamine couples with Fast Garnet GBC salt to produce a strong brownish-red color.

The data on the specificity (Table 2) showed that the color was also detected in some samples of breast milk. This result is in accord with the report [2] that human breast milk contains high γ-GTP activity. In a few samples of vaginal fluid, positive reaction was observed (Table 2). However, the color intensity was much weaker than that of seminal stains; when judged within 5 min, the color was almost negative. Therefore, it was easy to distinguish between semen and vaginal fluid. This problem is also the case for ACP test [6].

γ-GTP has been claimed to be much more stable than ACP [1–3]. This was also evidenced by our result that only one of 11 seminal samples, left at room temperature for 11–23 years, was negative (Table 1), showing that the present method is suitable for old seminal stains. In addition, the method is very simple and quick; more than 100 samples can be treated within 30 min. Therefore, we can recommend our method as a confirmatory test of the ACP method for identification of seminal stains.

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