Z. Rechtsmed. 83, 115—119 (1979)

Zeitschrift für
Rechtsmedizin

The LAP Test

A New Method for Identification of Seminal Stains by a Qualitative Color Reaction of Leucine Aminopeptidase

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Summary. A new simple method for identification of seminal stains is described. It employs a qualitative color reaction based on a histochemical technique for demonstration of leucine aminopeptidase (LAP), which is extremely abundant in human semen. The method herein reported (the LAP test) is quite suitable for medicolegal examination of seminal stains as a preliminary test.

Key words: Examination of stains, identification of semen – Identification of semen, leucine aminopeptidase – Identification of semen, preliminary test – LAP-Test

Zusammenfassung. Es wird eine neue einfache Methode zum Nachweis von Spermaspuren beschrieben. Sie benutzt eine qualitative Farbreaktion, die auf einer histochemischen Technik zur Darstellung der Leucin-Aminopeptidase (LAP) beruht, die im menschlichen Sperma besonders reichlich vorhanden ist. Die hier angegebene Methode (der LAP-Test) ist für die gerichtsmedizinische Samenspurenuntersuchung als Vorprobe („Suchreaktion") recht geeignet.

Schlüsselwörter: Spurenuntersuchung, Spermanachweis – Spermanachweis, Leucin-Aminopeptidase – Spermanachweis, Vorprobe – LAP-Test

The acid phosphatase test demonstrating the presence of large amounts of the enzyme in human semen has widely been used for medicolegal identification of seminal stains as a preliminary test [1], and the most commonly used method involves examining the development of a characteristic color [2]. However, various biological materials such as urine, vaginal fluids, feces, semen of monkey and dog, many vegetable extracts and colonies of *Staphylococcus aureus* also contain considerable amounts of acid phosphatase, which can cause the development of color though it develops much slower than with semen [3—7]. Hence, it is worthwhile to seek a more specific means of identifying human semen.

0044-3433/79/0083/0115/$ 1.00

Krampitz and Doepfmer [8] reported that human seminal plasma contains a high enzymatic activity of leucine aminopeptidase (LAP) hydrolyzing *L*-leucyl-*β*-naphthylamide. We have recently observed that LAP in ejaculated human semen is greatly in excess of that found in any other body fluid, animal semen, and juices of common vegetables and fruits [9]. This suggests that the determination of LAP in extracts of stains is applicable to medicolegal examination of semen. However, such a quantitative method is considered inadequate as legal proof.

We present here a simple method of recognizing LAP (the LAP test) as a screen for the presence of seminal stains. The test employed is a qualitative color reaction based on a histochemical technique of Burstone and Folk [10]. Wherever semen is present, LAP hydrolyzes the substrate *L*-leucyl-*β*-naphthylamide to release *β*-naphthylamine, which couples with the diazonium salt (Fast Garnet GBC) to form an insoluble red-pink azo dye.

Materials and Methods

Stains. Various human body fluids including semen, semen of some mammals, and expressed juices of vegetables and fruits which are likely to be encountered in forensic work were dropped or smeared on filter paper (Toyoroshi No. 2, Tokyo, Japan). They were allowed to dry at room temperature for a few hours and examined by the present method.

Known human seminal stains of various ages previously made on cotton gauze and stored dry at room temperature were also tested.

Reagents. Reagent I: 5 mg *L*-leucyl-*β*-naphthylamide · HCl was dissolved in 10 ml distilled water. This is stable for about 1 month in a refrigerator.

Reagent II: 20 mg Fast Garnet GBC was dissolved in 10 ml 0.2*M* Tris-HCl buffer (pH 7.1) immediately before use.

Procedure. A small piece of stained material was cut and placed on a hollowed glass plate. One drop of a mixture of Reagent I and Reagent II (1:1) was added onto the stain. A positive reaction was indicated by the development of a red-pink color. A distinctive color reaction occurring within 5 min was considered a very good indication of the presence of human semen. This reaction time was arbitrarily established by whole observation of the results.

Results and Discussion

Most human seminal stains caused the development of a red-pink color within 1 min after the mixture of the reagents had been dropped onto them, and some as early as about 30 s. The color gradually became intense with increasing time of the reaction, and all the human seminal stains examined exhibited an intense color within 5 min. However, weak color reactions began to appear in some fecal stains at reaction times between 3—5 min and in stains of cauliflower and mushroom at reaction times between 1—3 min. All the other samples gave no color reactions. The results examined at 5 min are summarized in Tables 1—4, which indicate that LAP is highly specific for human semen, and thus the positive LAP test provides strong evidence for the presence of semen. The present

Table 1. Results of the LAP test obtained from stains of various human body fluids

Human body fluid	No. tested	Positive	Negative
Semen[a]	50	50	0
Normal blood	12	0	12
Normal serum	40	0	40
Pregnancy serum[b]	9	0	9
Salive	20	0	20
Nasal discharge	6	0	6
Tear	6	0	6
Breast milk	6	0	6
Perspiration	10	0	10
Urine	40	0	40
Vaginal fluids[c]	30	0	30
Feces	35	13	22

[a] Collected from male volunteers by masturbation
[b] Collected from pregnant women during the third trimester of pregnancy
[c] Collected from women who had an abstinence period over 5 days. We are very grateful to Dr. K. Higashide, Obstetrics and Gynecology Clinic, Nagoya University Hospital for his kind cooperation

Table 2. Results of the LAP test obtained from seminal stains of various animals

Animal semen	No. tested	Positive	Negative
Boar[a]	3	0	3
Bull[a]	3	0	3
Dog[b]	4	0	4
Horse[c]	1	0	1
Rhesus monkey[d]	1	0	1

[a] Offered from the Livestock Centre of Aichi Prefecture, Okazaki
[b] Offered from a veterinary surgeon
[c] Offered from a stock farm
[d] Offered from the Japan Monkey Centre, Inuyama

observations are mainly compatible with the results of our previous LAP quantitation in the same samples [9].

Table 5 shows the results of the test on human seminal stains of various ages examined at a reaction time of 5 min. All the stains under 1 year old gave a strong color reaction. A recognizable color reaction was seen in all but one stain between 2—10 years old. The data show that this method can be used for demonstration of aged seminal stains within 10 years after stain formation.

It should be emphasized that no false positive LAP reactions were found in vaginal fluids, giving a distinct advantage in examination of vaginal swabs in

Vegetable	No. tested	Positive	Negative
Cabbage	2	0	2
Carrot	2	0	2
Cauliflower	5	5	0
Celery	2	0	2
Cucumber	2	0	2
Egg plant	2	0	2
Green pepper	2	0	2
Leek	2	0	2
Lettuce	2	0	2
Mushroom	5	5	0
Onion	2	0	2
Parsley	2	0	2
Radish	2	0	2
Spinach	2	0	2
Sweet potato	2	0	2
White potato	2	0	2

Table 3. Results of the LAP test obtained from stains of vegetable extracts

Fruit	No. tested	Positive	Negative
Apple	2	0	2
Grape	2	0	2
Grapefruit	2	0	2
Lemon	2	0	2
Loquat	2	0	2
Melon	2	0	2
Orange	2	0	2
Peach	2	0	2
Pear	2	0	2
Persimmon	2	0	2
Pineapple	2	0	2
Strawberry	2	0	2
Tomato	2	0	2
Watermelon	2	0	2

Table 4. Results of the LAP test obtained from stains of fruit extracts

actual cases of sexual assault. However, the test seems unsuitable for examining anal swabs since a weak positive reaction occurred in some fecal stains.

The LAP test presented here is now in routine use for screening seminal stains and has replaced the acid phosphatase test in our laboratory. The simplicity of the technique and the high specificity of the reaction make it also recommendable for the routine practice of any forensic science laboratory.

Table 5. Results of the LAP test obtained from human seminal stains of various ages

Age of stain	No. tested	Positive	Negative
1 day	20	20	0
1 week	20	20	0
2 weeks	20	20	0
3 weeks	20	20	0
1 month	20	20	0
2 months	6	6	0
3 months	6	6	0
6 months	10	10	0
1 year	11	11	0
2 years	1	1	0
5 years	4	3	1
8—10 years	3	3	0
13—15 years	14	8	6
19—22 years	12	5	7

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Received December 4, 1978