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Deoxyribonuclease I (DNase I) Typing from Semen Stains: Low Enzyme Activity in Vaginal Fluids Does Not Interfere with Seminal DNase I Typing from Mixture Stains

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ABSTRACT: We describe the use of deoxyribonuclease I (DNase I) polymorphism for individualization of semen in body fluid stain mixtures, as a means of providing new and more useful information to practicing forensic biologists as a genetic marker. We have already reported that human DNase I isozyme patterns from different subjects are classifiable into ten groups. Isoelectric focusing of DNase I isozymes on polyacrylamide gel (IEF-PAGE, pH 3.5 to 5) was accomplished using a 0.5 mm thick gel. Pretreatment of semen samples with neuraminidase enhanced the isozyme band resolution and sensitivity. Activity detection using the dried agarose film overlay (DAFO) procedure was reliable, sensitive and simple, with high resolution, and the phenotypes of DNase I were determined in semen stains of about 0.3 μL stored at room temperature for up to a year in most of the samples tested. The DNase I types in semen stains were correlated with the types found in the corresponding blood and urine samples, although most of the vaginal fluid samples had no typable DNase I activity. This is considerably advantageous for seminal individualization from body fluid mixture stains in criminal cases. An evaluation of DNase I typing by IEF-PAGE and DAFO was also performed on casework samples submitted to our laboratory, and the results showed that DNase I was expected to be one of the most useful individualization marker of semen in practical application.

KEYWORDS: criminalistics, deoxyribonuclease I (DNase I), dried agarose film overlay (DAFO) method, isoelectric focusing, semen identification, single radial enzyme diffusion (SRED) method, vaginal fluid

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Deoxyribonuclease I (DNase I, EC 3.1.21.1) is an enzyme that preferentially attacks double-stranded DNA and produces 5′-phosphodinucleotides and 5′-phosphooligonucleotides by endonucleolytic cleavage [*1,2*]. DNase I activity in human tissues and body fluids has been measured: urine showed the highest activity; pancreas, kidney, submandibular gland, liver, spleen, placenta, heart, thymus, semen, saliva, and breast milk gave the detectable activities; brain, lung, erythrocytes, and leukocytes showed no activity [*3,4*]. The presence of DNase I activity outside digestive system suggests that the enzyme has another biological function in addition to its digestive role. Recently, the amino acid sequence of DNase I was determined by proteochemical [*5,6*] and DNA sequence [*7*] analyses.

Genetic polymorphism of DNase I has been detected in body fluids and tissues by isoelectric focusing, and ten phenotypes of DNase I are known to be controlled by four co-dominant alleles, *DNASE1*1*, **2*, **3*, and **4* [*8*] giving rise to ten phenotypic isozyme patterns. The frequencies of these alleles calculated in a Japanese population were 0.552, 0.436, 0.010, and 0.002, respectively [*5,9*].

The most common genetic markers used so far for individualization of semen in semen stains and mixed body fluid stains have been the ABO and phosphoglucomutase-1 (PGM1) systems. Recently, we have reported that transferrin (TF) typing is also very useful for discrimination of semen in crime-scene materials [*10,11*]. However, there is a need for a new and more effective system of discrimination, in addition to these systems.

DNase activity has been detected in semen [*12*], and Sawazaki et al. [*13*] have described a preliminary survey assessing the correlation among serum, urinary and seminal DNase I phenotypes. Also, Yasuda et al. [*14*] have reported that seminal and urinary DNases I show high enzymological and proteochemical similarity. Consequently, the present authors have pursued further studies to evaluate the use of seminal DNase I in a case work situation. In this line of work, semen stains are encountered not only in the form of whole uncontaminated semen but also as mixtures with other body fluids. The most common contaminant of semen in this context is vaginal drainage after sexual intercourse. Although semen is sometimes found mixed with saliva, urine and other contaminants during the course of crime investigation, it is not practical to investigate semen stains other than the most commonly encountered forms. Consequently, this paper describes experiments carried out on stains of pure semen and semen/vaginal fluid mixtures.

Materials and Methods

Preparation of Samples for Analysis

Collection of semen and blood samples—Healthy male donors (110) supplied samples obtained by mastervation or by withdrawal during sexual intercourse. The ejaculate was collected in clean, wide-mouthed plastic containers with screw caps. These samples were presented at the laboratory within 12 h of collection and frozen immediately at −80°C until use [*11*]. Serum samples were prepared from venous blood collected from healthy donors. Blood was centrifuged and the supernatant was stored at −80°C until use.

Collection of vaginal swabs—During examinations for gynecological insufficiency, 25 vaginal swabs taken during and outside the menstrual period were obtained from the vaginal fornix. These swabs were stored immediately at −80°C until required for testing. The samples were taken on standard wooden-spilled cotton wool swabs.

Preparation of semen stains—The experimental design of the survey required 3 to 14 separate stains of whole semen to be made from each sample. Each semen sample (1 mL) was mixed thoroughly after thawing and then pipetted onto clean cotton sheeting. The stains so made were approximately 30 cm^2 in area. These were hung up to air-dry,

divided into five separate groups and stored in separate paper envelopes at −20°C, +4°C, room temperature, +37°C and in a humid chamber at +37°C, until required for DNase I grouping [*13*].

Preparation of vaginal swab extracts—The cotton wool tip of one swab from each female donor was removed, and then extracted in 2 mL of 0.1% (w/v) bovine serum albumin containing 2 m*M* $CaCl_2$. This was carried out in the upper compartment of the extraction device described previously [*15*]. After centrifugation, the swab extract was concentrated to about 200 μL using a Minicon B15 concentrator (W.R. Grace & Co., Danvers, MA) and divided into several portions. One of these was tested for acid phosphatase activity by dropping onto a clean filter paper, followed by one drop of the phosphatase reagent used for screening [*16*]. The remainder was subjected to determination of DNase I activity using the single radial enzyme diffusion (SRED) method [*3,4*], and then analyzed using polyacrylamide gel isoelectric focusing (IEF-PAGE) and the dried agarose film overlay (DAFO) method [*9,19,20*], as described in the following section.

Extraction of DNase I from semen stains—A piece of semen-stained cotton sheeting measuring 3 by 3 mm (equal to about 3 μL of liquid semen) and 10 μL of each solution for DNase I extraction were employed for each test according to the micro-extraction method described in our previous paper [*15*]. As the diluent for the extracts, 1% (w/v) bovine serum albumin containing 20 m*M* $CaCl_2$ was used in order to prevent DNase I denaturation. The enzyme was more stable in the presence of Ca^{2+}, as described in our previous paper [*6*].

Preparation of urine samples—Urine samples were concentrated, dialyzed and finally freeze-dried, as described previously [*17,18*]. A 0.1% (w/v) solution of the freeze-dried material, corresponding to about 10-fold-concentrated urine, was used for DNase I typing.

Method of DNase I Typing

IEF-PAGE was performed in thin-layer gel, pH 3.5-5, as described previously [*13*]. Gels measuring 0.5 (thickness) by 90 (width) by 120 (length) mm were prepared using the following materials: 1.4 mL acrylamide-bisacrylamide (19.4%, w/v; 0.6%, w/v), 1 mL distilled water, 2.3 mL sucrose-glycerol (20%, w/v; 10%, v/v), 280 μL Ampholine 3.5-5 (Pharmacia LKB, Uppsala, Sweden), 5 μL *N,N,N′,N′*-tetramethylethylenediamine, and 40 μL 1.2% (w/v) ammonium persulfate. Wicks were formed from strips of filter paper and soaked in the electrode solution: 1.0 *M* H_3PO_4 at the anode and 2% (v/v) Ampholine 5-7 (Pharmacia LKB) at the cathode. Each sample was applied to the gel with a plastic applicator at a distance of 20 mm from the cathode wick. A Multiphor apparatus (Pharmacia LKB) was employed to run the gel at V_{max} 1000 V, I_{max} 10 mA, and P_{max} 3 W for 4 h under cooling at +12°C.

Semen diluted 1:4 with distilled water and extracts from semen stains were treated with an equal volume of 5 units/mL *Clostridium perfringens* neuraminidase (Sigma, Type V, St. Louis, MO) before electrophoresis [*13*]. Five microliters of the digest was used for IEF-PAGE analysis. Blood and urine samples were analyzed using the previous methods [*8,9,19*].

Visualization was achieved using the DAFO method [*9,19,20*] based upon the fact that ethidium bromide produces fluorescence only with large DNA molecules, but not with degraded DNA digested by DNase I. The reaction mixture consisted of 0.05 mg of ethidium bromide and 0.1 mg of salmon testis DNA (type III, Sigma) per 1 mL of 100 m*M* sodium cacodylate buffer, pH 6.5, which contained 0.2 m*M* $CaCl_2$ and 20 m*M* $MgCl_2$. The thin agarose film was prepared as follows: To the reaction mixture, an equal volume

of 2% (w/v) molten agarose was added, mixed and poured onto a horizontal Agafix plastic sheet (Wako Pure Chem. Ind. Ltd., Osaka, Japan) to a thickness of about 2 mm. About 5 mL of the mixed solution was sufficient to prepare an agarose film measuring 5 by 7.5 cm. After solidification at room temperature, the agarose gel was dried completely in an incubator at about 50°C to produce a dried agarose film sheet. After the IEF-PAGE run, the film sheet was placed on top of the focused gel in full contact. The gel was incubated in contact with the film sheet at 37°C, and the progress of DNase I action was observed under UV light (312 nm). Normally, sharp dark bands corresponding to the isozymes appeared within 1 to 2 h. After incubation for optimal development, the film sheet was removed from the gel and washed with distilled water, and then observed or recorded photographically.

Method of DNase I Quantitation

DNase I was assayed by the SRED method [*3,4*]. Each assay gel plate was prepared as follows: 5.1 mL 100 m*M* cacodylate buffer, pH 6.5, containing 20 m*M* $MgCl_2$ and 2 m*M* $CaCl_2$ was placed in a test tube; 0.27 mL 1% (w/v) salmon testis DNA (type III, Sigma) and 54 μL 0.5% (w/v) ethidium bromide solution were added and mixed; 5.4 mL of 2% (w/v) molten agarose was added and poured onto a horizontal Agafix sheet; the final volume (10.8 mL) was sufficient to prepare a gel plate measuring 7.5 by 13.5 cm and 2 mm thick. After solidification, a row of cylindrical sample wells (radius, r_0: 1.0 mm) with centers 15 mm apart was punched in the gel; a 7.5 by 13.5-cm gel plate accommodated 32 (4 by 8) sample wells. Unknown samples (2 μL) and serial dilutions of purified urine DNase I of known activity were placed in the wells. After incubation of the gel plate in a moist chamber at 37°C for 20 h, a circular dark zone with a radius, r_s, was observed on an ultraviolet transilluminator (312 nm) as DNase I diffused radially from the well into the gel and digested the substrate DNA. A standard curve was constructed by plotting Log_{10} DNase I activity against the diffusion radius ($r_s - r_o$).

Other chemicals used were of the purest grade available commercially.

Results

Quantitation of seminal DNase I and liquid semen survey

The levels of seminal DNase I activity were estimated from 110 samples by the SRED method [*3,4*]. The mean value of the seminal enzyme activity was determined to be (28 ± 4.0, mean ± S.D.) × 10^{-3} units per mg protein (range, 11-81 × 10^{-3}), which was estimated simultaneously in the semen volume to be 0.25 ± 0.06 units per mL. This mean value agreed with another study [*4*].

The DNase I type of 110 Japanese male donors was determined from both blood (or urine) and semen samples, and the results agreed with the donor's DNase I types in each case, as described for our preliminary survey [*13*]. These samples were groupable into 3 types: type 1, 35; type 1-2, 57; type 2, 18. The IEF-PAGE patterns of seminal DNase I in each type were almost identical to those of the serum and urinary enzyme (Fig. 1). This supposition was supported by the finding that a mixture of serum (or urine) and semen type 1 (type 2) gave a single main isozyme band indistinguishable from that of type 1 (type 2) serum (or urine) on the gel.

Effects of solutions on DNase I extraction

Reducing reagents (2-mercaptoethanol and dithiothreitol) at different concentrations and physiological saline had no favorable effect on extraction of DNase I from the aged

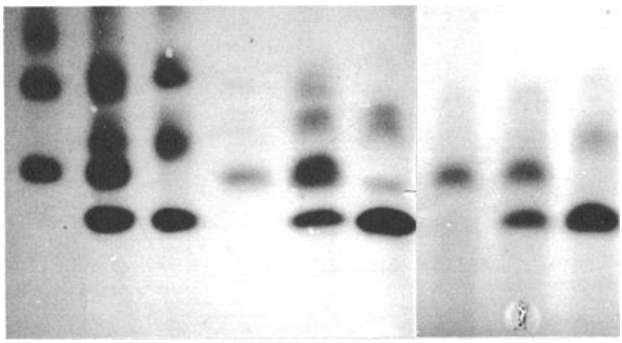

FIG. 1—*Isoelectric focusing (pH 3.5-5) patterns of three common DNase I phenotypes from desialylated urine (lanes 1, 2 and 3 from left to right), semen (lanes 4, 5 and 6) and serum (lanes 7, 8 and 9) samples detected by activity staining using the DAFO method (see Materials and Methods). Lanes 1, 4 and 7, type 1; lanes 2, 5 and 8, type 1-2; lanes 3, 6 and 9, type 2. Anode is at the top.*

stains, which were kept for 6 weeks at room temperature. The effects of distilled water containing 20 m*M* $CaCl_2$ and 1% (w/v) bovine serum albumin, several 0.05-2.0% (v/v) detergents (3-[(3-cholamidopropyl)dimethylammonio-1-propanesulfonate (CHAPS), Tween 20, Tween 80, Nonidet P-40, and Triton X-100) and urea at different concentrations for extraction of DNase I were also examined. The 1% (w/v) bovine serum albumin containing 20 m*M* $CaCl_2$ was the most effective for enzyme extraction, and therefore, it was employed for each subsequent extraction throughout this study. Urea at 0.3-1 *M* was also considerably effective for extraction (data not shown).

Stability study on semen stains

Details of the results are shown in Table 1. The possibility of typing DNase I isozyme in semen stains aged up to 13 or 52 weeks was examined under five different conditions. Every sample stored at −20°C, +4°C, room temperature and +37°C was typable without false interpretation after dry storage for up to 27 weeks. However, several samples kept at room temperature for 52 weeks were slightly difficult to type because of faint and indistinct band formation and others were incorrect or inconclusive. It was surprising that the semen stains stored at +37°C in humid conditions were typable correctly after 13 weeks. Some samples of the results obtained from aged semen stains stored under these conditions are shown in Fig. 2.

Detection limits for semen stains

We determined the detection limits for semen stains. Under four dry storage conditions, except at −20°C, a slow decrease in the enzyme activity was observed during the course of storage. The detection limit had dropped to about 1/5 of the level for fresh stains after 52 weeks of dry storage at room temperature (Table 2), and similar results were obtained after dry storage at +4°C and +37°C. The amount of semen stain required in order to type heterozygotes (type 1-2) was slightly more than that for homozygotes (type 1 and type 2). It proved possible to determine DNase I types from 52-week-old stains made from about 0.3 μL of semen and kept at room temperature.

TABLE 1—*DNase I stability study of 69 laboratory prepared semen stains maintained under various conditions.*[a]

Weeks	−20°C			+4°C			room temp.			+37°C			humid, +37°C		
Phenotype (Number)	1 (2)	1–2 (2)	2 (2)	1 (5)	1–2 (5)	2 (4)	1 (10)	1–2 (14)	2 (4)	1 (4)	1–2 (5)	2 (3)	1 (3)	1–2 (3)	2 (3)
1 (day)	2	2	2	5	5	4	10	14	4	4	5	3	3	3	3
4	2	2	2	5	5	4	10	14	4	4	5	3	3	3	3
13	2	2	2	5	5	4	10	14	4	4	5	3	3	3	3
27	2	2	2	5	5	4	10	14	4	4	5	3	NT[b]	NT	NT
52	2	2	2	5	5	4	9	12	4	NT	NT	NT	NT	NT	NT

[a]The values indicate the number of conclusive results. There were no incorrect, inconclusive or negative results over this time period.
[b]Not tested, because the samples were used up.

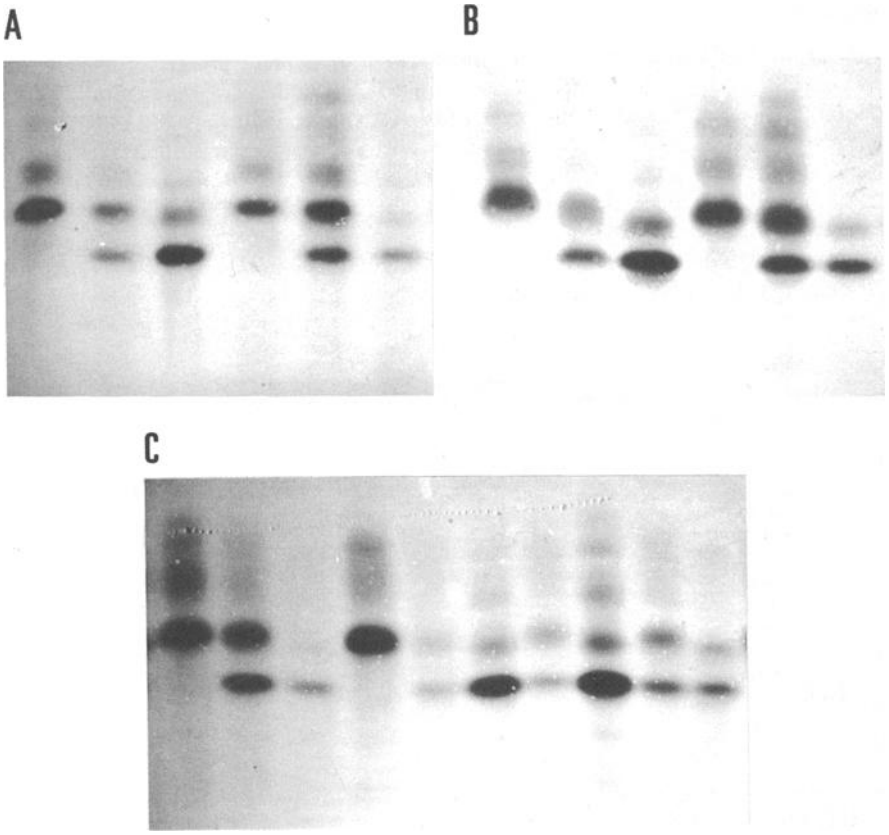

FIG. 2-A,-B,-C—*Isoelectric focusing (pH 3.5-5) patterns of DNase I types from semen stains stored at room temperature for 27 weeks (A: lanes 1 and 4, type 1; lanes 2 and 5, type 1-2; lanes 3 and 6, type 2 from left to right), semen stains stored at room temperature for 52 weeks (B: lanes 1 and 4, type 1; lanes 2 and 5, type 1-2; lanes 3 and 6, type 2), and semen stains stored at 37°C for 27 weeks (C: lanes 1 and 4, type 1; lanes 2, 5, 7 and 9, type 1-2; lanes 3, 6, 8 and 10, type 2) detected by activity staining using the DAFO method. Anode is at the top of each panel.*

TABLE 2—*Minimum detection limits for semen stains of different DNase I types stored at room temperature.*[a]

	DNase I phenotype		
Weeks	1	1–2	2
1 (day)	40	50	40
4	80	100	80
13	80	100	80
27	120	180	120
52	250	250	250

[a]nL of liquid semen required for DNase I typing from the stains. The values were calculated by the size of tested semen stains (3 by 3 mm, contained approximately 3 μL of liquid semen), and the volumes of extraction solution (10 μL), the added sialidase solution (equal volume to the extract) and the applied sample solution (5 μL) for IEF-PAGE separation, and the DNase I-typable maximum dilution of the finally obtained IEF-PAGE samples. The values were averaged from the results of more than four different semen samples in all cases.

Vaginal swab survey

Semen-free vaginal swabs were obtained from 10 women throughout the menstrual cycle. Additionally, semen-free swabs from 3 other women taken at random, were examined (a total of 25 swabs being analyzed). Activity was not detected in 92% (23/25) of swabs by the SRED technique, but when DNase I was detected, their activities were always low ($<0.1\text{-}0.2 \times 10^{-3}$ units per swab or $< 6 \times 10^{-3}$ units per mL of vaginal secretion, i.e., 1/40 lower than semen per volume), and the vaginal DNase I type could be determined from only two cases. This showed that the enzyme contents of most vaginal fluids seemed to be lower than the detection limits for enzyme typing. No relationship between enzyme activity and the menstrual cycle was found.

TABLE 3—*DNase I types in items from casework.*

Items		AP[a]	Sperm[b]	Blood-stained	Vaginal epithelium[b]	DNase I-type	DNase I-type[c]	
							Victim	Suspect
Case 1	Swab of vaginal material	+	+	−	+	1–2	1	1
Case 2	Swab of vaginal material	+	+	−	+	1	1–2	1
Case 3	Swab of vaginal material	+	+	+	+	1–2	2	1–2
Case 4	Swab of vaginal material	+	+	−	+	1–2	1	1–2
Case 5	Swab of vaginal material	+	+	+	+	1	2	1
Case 6	Semen stains	+	+	−	+	1–2	2	1–2
Case 7	Semen stains	+	+	−	−	1	2	NT[d]

[a]Acid phosphatase activity: +, positive; −, negative.
[b]Detected microscopically: +, present; −, not present.
[c]Determined from serum or urine samples.
[d]Not tested.

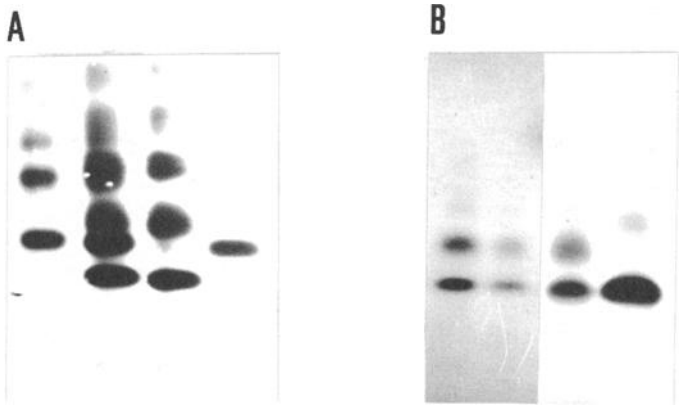

FIG. 3-A,-B—*DNase I typing in items from casework (cases 2 and 3 in Table 3). A [case 2]: lane 1, suspect's urine (type 1); lane 2, victim's urine (type 1-2); lane 3, control urine (type 2); lane 4, swab extract (type 1) from left to right. B [case 3]: lane 1, swab extract (type 1-2); lane 2, 1:2-diluted swab extract (type 1-2); lane 3, suspect's serum (type 1-2); lane 4, victim's serum (type 2) from left to right. Anode is at the top.*

See the text and Table 3 for further details.

Survey of items from casework

Details of a limited number of items from routine casework in our laboratory are given in Table 3. Unfortunately, time had elapsed between the offense and the swab being taken, and other items of information on the victims, suspects, and crime scenes were unknown in each case. A case 1 swab from a woman of type 1 gave a band pattern that was interpreted as type 1 mixed with type 2 or type 1-2 semen. The type 1 suspect in this case would therefore have been deviated as the semen donor. A case 6 semen stain contaminated with vaginal secretion of a type 2 woman gave a band pattern that was interpreted as type 2 mixed with type 1 or type 1-2 semen, and therefore, the type 1-2 suspect could not be excluded as the perpetrator. The analyzing results obtained from the cases 2 and 3 are shown in Fig. 3.

Discussion

The mean value of seminal DNase I determined from 110 samples was 0.25 ± 0.06 units/mL. This value was equal to that estimated previously by ourselves [*13*] and other workers [*4,14*].

DNase I types are very valuable in terms of discrimination potential [*8,9*], and the enzyme levels in semen are reported to be sufficiently high for detection by our typing method [*9,13,19*], and therefore no concentration step is required prior to analysis. The combined technique of IEF-PAGE and activity staining using the DAFO method produced more than a 100-fold increase in sensitivity as compared with conventional detection using immunostaining [*8,19*], and we were able to type DNase I from all the seminal samples (range of DNase I content: 0.16 to 0.34 units/mL semen, because we succeeded in overcoming a major quantitative barrier preventing isozyme analysis.

Our previous work [*13*] showed that there was a good correlation between the DNase I types of semen and corresponding blood and urine samples. The present work extends these previous findings. Consequently, semen and blood samples from a total of 110 individuals (type 1, 35; type 2-1, 57; type 2, 18) have now been examined. No examples of non-correlation in DNase I typing between corresponding samples have been found. Moreover, all the individuals showed sufficiently detectable DNase I isozyme bands in the semen. The presence of DNase I activity in semen from 3 men with azoospermia and 3 with oligospermia, tested and the absence of the activity in sonicated 50 μL lysate from 10^6 spermatozoa (unpublished data) indicate that the enzyme is useful in instances where no sperm are present in the semen, and these findings also indicate that the enzyme does not originate from sperm. Yasuda et al. [*14*] have reported that the prostate is one of the source tissues of the seminal enzyme.

The surveys described in the present paper were designed to simulate some of the problems encountered by the practicing forensic biologist in a casework situation, where one is required to individualize body fluids and their mixtures using genetic markers. In this respect, the surveys were designed to test the validity of the DNase I typing technique in these situations.

The influence of storage conditions on the time limit for detection of DNase I types was examined (Table 1). Every sample stored at −20°C, +4°C, room temperature and +37°C was typable without false interpretation after at least 27 weeks of dry storage. However, temperature and air humidity during storage were the major constraints affecting detectability. In another experiment, semen (1 μL) of type 2 was added to vaginal swabs from a type 1 donor, and incubated in a humid chamber at +37°C to approximate *in vivo* conditions. Pieces were removed from the swab every 2 h and typed. The volume of semen was chosen in order to produce band patterns of optimum enzyme activity, and it was found that all of this activity appeared sufficiently until 12 h (data not shown). From this work, we feel that the length of time that has elapsed between intercourse and

swabbing should not be an important criterion in deciding whether DNase I typing should be attempted, because of the highly stable nature of DNase I isozyme.

According to our experience, most of the proteins such as TF and C6 in aged stains were difficult to extract with water or saline alone and redissolved [*10,11,15*]. Therefore we examined the best solution to use for this purpose. A 1% (w/v) bovine serum albumin containing 20 m*M* $CaCl_2$ or 0.3-1 *M* urea was the most effective among several detergents and other solutions tested, improving and increasing the efficiency of detectability of seminal DNase I types by two- or three-fold as compared with other solutions.

We also determined the limits of detection of semen stains. Under five storage conditions, except at −20°C, a slow decrease in the activity of DNase I was observed during the course of storage. Though the detection limit had dropped to 1/5 of the level for fresh stains after 52 weeks of dry storage at room temperature, it proved possible to determine DNase I types from 52-week-old stains made from about 0.3 μL of semen (Table 2). The amount of semen stains required in order to type heterozygotes was slightly larger than that for homozygotes. In practice, stains of this volume would be applicable in routine casework.

In most of the semen-free swabs tested, the DNase I types were not established, because of very low concentration of the vaginal enzyme. These results, however, showed that considerable care might be needed when typing semen and interpreting the band patterns on vaginal swabs using DNase I polymorphism. Investigation of vaginal swabs for seminal DNase I showed that valuable information can be obtained irrespective of the woman's type and that the results are attributable to the semen present, that is, the woman's DNase I type may not mask the seminal DNase I type in all pairings. From these findings, the use of the ABO, PGM 1 and TF blood group systems for individualization of semen in body fluid mixtures may be superseded by the use of seminal DNase I as a genetic marker. Of course, we don't say that DNase I should supplant ABO and other genetic markers rather than complement them.

Five instances of DNase I typing of actual case-swabs submitted to the laboratory were tested (Table 3). In cases 2 and 5, although the victims were types 1-2 and 2 respectively, we could not detect the type 2 component of the victims, and the type 1 component alone was evident on the swabs. In the case 3 swab, the detected type 1 component was attributable to semen which could have originated from a type 1 or 1-2 man, because the victim was type 2. In the case 4 swab, the detected type 2 component was attributable to semen which could have originated from a type 2 or 1-2 man, because the victim was type 1. From these findings and the very low levels of vaginal DNase I activity described above, it is likely that DNase I type determined in most of the mixture stains of seminal and vaginal secretions is seminal DNase I type alone. For example, in the case 3 swab, we detected types 1 and 2 components, and this was attributable to semen which could have originated from a type 1 or 1-2 man since the victim was type 2, although it was highly probable that the semen was derived from a type 1-2, and not a type 1 man (Fig. 3). This is in striking contrast to the ABO [*16*], PGM1 [*22*] and TF [*11*] typings, in which most vaginal swabs show mixtures of the victim's and suspect's types, and shows the great advantage of DNase I typing in semen/vaginal secretion mixtures. However, even if vaginal fluid enzyme levels were probably undetectable, it is not wise to discount the woman's type in analyzing swab evidence unless there is independent evidence that semen predominates the samples.

It has been reported that a combination of IEF-PAGE and subsequent immunodetection is very important for crime investigations of genetic markers detected in body fluids [*5,8,15,23–26*]. We used this technique in the present study, but could not identify the correct DNase I type from both fresh seminal fluids and semen stains, because of the 1/100 lower sensitivity of immunostaining compared with DAFO activity staining. This demonstrates that successful and effective detection is closely dependent on the

methodology used rather than the degradation of enzyme protein with time, as pointed out previously [*10,11*].

DNA polymorphism can now be applied to the individualization of semen, bearing in mind its high power of discrimination, although its validity has been questioned in court [*27,28*]. Therefore, conventional genetic markers like DNase I, which can be detected by the traditional, but well approved and well established methods with an unquestioned genetic basis, are now also very important for criminal investigation of semen samples.

In conclusion, DNase I isozyme polymorphism is a particularly useful marker of individuality in forensic science, since the discriminating power of the *DNASE1* locus is high for the isozyme phenotypes revealed by IEF-PAGE followed by DAFO detection [*8,9*]. Furthermore, the isozymes are present in a variety of body fluids without vaginal secretions, and enzyme activity [*4*] is retained for prolonged periods in stains recovered from crime scenes.

Acknowledgment

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