TECHNICAL NOTE

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DNA Typing of Azoospermic Semen at the D1S80 Locus*

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ABSTRACT: The examination of azoospermic semen poses a special problem for the forensic scientist. Both serologic and RFLP methods may result in inconclusive results. PCR analysis is known to have an advantage in the evaluation of variably degraded, small quantities of DNA. This investigation addresses the feasibility of detecting the DNA profiles of azoospermic males in cases of suspected rape by the use of PCR amplification of the VNTR locus D1S80. DNA profiles were produced from aspermic semen samples from six vasectomized males. Two mixed postcoital vaginal samples containing azoospermic semen from two of the vasectomized males were also obtained and both revealed the combined profiles of the azoospermic semen donors and the vaginal epithelial donors. All cases resulted in an allelic banding pattern of the donor semen matching the respective blood/saliva standard.

KEYWORDS: forensic science, rape, sexual assault, azoospermia, DNA typing, restriction fragment length polymorphism, polymerase chain reaction, variable tandem number repeat, semen, D1S80

Sexual assault is a major component of violent crime in the United States. A thorough forensic rape investigation requires a clinical or postmortem examination for the presence of semen in a victims's mouth, vagina, anus, external genitalia, adjacent body surface area, or clothing. Because spermatozoa can remain in the vaginal vault in a motile form for greater than 8 h and in a nonmotile form for approximately 24 h, semen often provides the best information for inculpating or exculpating a suspect (1). Traditional forensic analyses of semen include serologic tests which are characteristically accompanied by the microscopic identification of spermatozoa. Serologic identification of semen in the appropriate specimen only indicates penile intravaginal (intrarectal or intraoral, *mutatis mutandis*) intromission and ejaculation thereby satisfying one element of the criminal definition of rape, i.e., penetration (2). Body fluid mixtures are problematic because serologic markers from the contributing individuals may not always be discernable. With the advent of DNA profiling technology, a higher discriminating power of individual specificity can be reached. As with serological methods, DNA analyses of semen can still generate unique problems (3–5).

Azoospermic semen is periodically encountered in the investigation of rape cases with varying analytic outcomes. An azoospermic individual has virtually a total absence of sperm in ejaculate semen due to either congenital defects, successful vasectomy, or other environmental or psychogenic factors (6). Sperm supplies the vast majority of DNA in an ejaculate sample; therefore, an azoospermic individual has much less seminal DNA for genotypic analysis. The amount of DNA/mL in the ejaculate sample of a spermic individual is approximately 450 μg from the spermatozoa with only 30 μg contributed by the leukocytes and epithelial cells (7). Thus, in an azoospermic individual, the DNA content approximates only 6.3% of that seen in a spermic individual.

DNA analysis using RFLP is a well-known method for characterizing individuals based on genetic profiles. Although RFLP has a high discrimination power, a minimum of 50 ng of high molecular weight DNA must be available to obtain interpretable results (8). Because only small amounts of variably degraded or azoospermic seminal fluid may be recovered in evidentiary samples, other techniques such as PCR may be useful for evaluation. PCR DNA amplification technology is rapid, sensitive and requires only small quantities (2.5–5.0 ng) of lower molecular weight (< 2 kb) DNA (4). Azoospermic semen from vasectomized males using RFLP analysis and PCR analysis of the HLA-DQ locus has undergone laboratory investigation with encouraging results (9). The VNTR D1S80 locus is highly polymorphic, offering discrete allelic resolution at an affordable cost. DNA fragments differing by less than 10 base pairs can be identified using this method (10,11). This study illustrates the use of the D1S80 VNTR allelic locus in evaluating azoospermic seminal stains.

Methods and Materials

Samples

"Neat" (unmixed) seminal fluid samples from six vasectomized volunteers were stained onto clean cotton swatches or cotton swabs. Seminal fluid samples were not quantified prior to cloth staining.

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Two separate postcoital mixed vaginal samples containing semen from two of the vasectomized individuals were also collected on clean cotton swabs. Microscopical examination of semen from each male participant confirmed the absence of sperm and the presence of leukocytes and epithelial cells (standard Hematoxylin and Eosin stain; 100 HPF/1000X,OI); (7).

Extraction/Quantification

Genomic DNA samples from either a 2-cm square cotton swatch or a single cotton swab were isolated by differential organic extraction, resulting in the following two fractions: fraction one containing all isolated nucleated cells other than spermatozoa and fraction two which would contain only spermatozoa, if present within the sample (3). After organic extraction, DNA was recovered utilizing the Microcon™ microconcentrator (Amicon, Inc., Beverly, MA) or using an ethanol precipitation method (8). The DNA was quantified by the Quantiblot™ DNA hybridization method developed by Walsh et al. (12).

Amplification

The extracted genomic DNA (3.5 ng) was amplified using the AmpliFLP™ D1S80 PCR kit with the GeneAmp PCR System 9600 as per manufacturer's instructions (Perkin Elmer Corp., Norwalk, CT). The sequences of the two oligonucleotide primers are: 5′-GAAACTGGCCTCCAAACACTGCCCGCCG-3′ and 5′-GTCTTGTTGGAGATGCACGTGCCCCTTGC-3′. They flank the short tandemly repeated DNA sequences at the D1S80 locus producing fragment length polymorphisms that compose different alleles. The D1S80 locus consists of a 16 base pair repeat with 27 known alleles consisting of 14 and 16–41 repeats as delineated by the AmpliFLP D1S80 allelic ladder. These allelic products range in size from 369–801 bp (10).

Electrophoresis/Detection

Ultrathin high resolution vertical polyacrylamide gel electrophoresis (PAGE) using Gene Amp® detection gel cast on gel bond was utilized for separation and detection of the alleles, 5 uL of amplified product DNA was analyzed. The gels were electrophoresed for approximately 2 h and 20 min at 1000 V utilizing 0.5% TBE (0.45 M Tris-HCL pH 8, 0.45 M boric acid and 1 mM EDTA) as the running buffer. Specific banding patterns were visualized by a simple silver staining method using the Promega DNA Silver Staining System developed by Bassam et al. (13). Development time varied to allow for optimal visualization of multiple bands in mixture samples.

Results and Discussion

Quantiblot analysis of extracted DNA revealed quantities suitable for amplification in fraction one but not fraction two from all specimens. Semen obtained from the six azoospermic individuals revealed discrete allelic banding patterns in fraction one; (Fig. 1, left panel). These samples were compared with their corresponding blood/salivary standards yielding consistent profiles (Fig. 1, right panel). Two mixed postcoital vaginal swabs containing azoospermic semen were examined revealing banding patterns in fraction one identical to the patterns from the blood/salivary standards of the two contributing individuals (Fig. 2). The left panel shows mixture one (1) in which the profiles are clearly discernable because the male is homozygous 18,18 whereas the female is

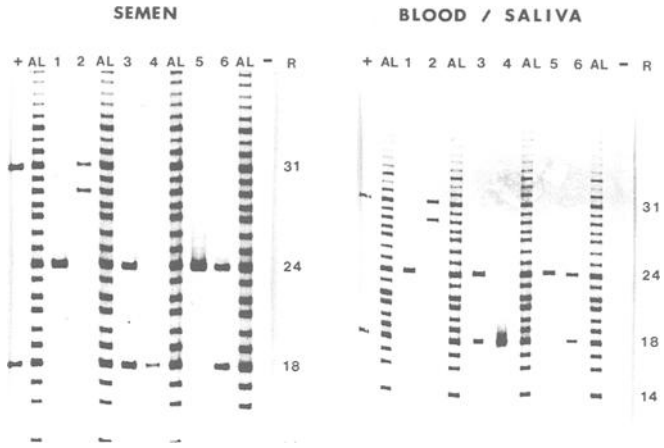

FIG. 1—*Vertical polyacrylamide gels displaying D1S80 genotypes of semen (left) and blood or saliva standards (right) from six vasectomized individuals (1–6). (+) = positive control with known genotype 18,31; AL = allelic ladder; (−) = no DNA (negative) control; R = number of repeats. Individuals' (1–6) genotypes are: 1–24,24; 2–29,31; 3–18,24; 4–18,18; 5–24,24; and 6–18,24.*

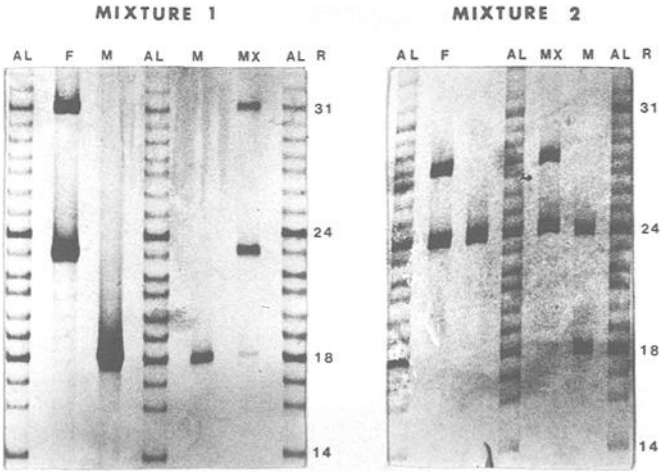

FIG. 2—*Polyacrylamide gel electrophoresis of two mixed postcoital vaginal samples containing azoospermic semen. AL = allelic ladder; M = male standard; F = female standard; MX = postcoital mixture; R = number of repeats. Mixture 1 genotypes are: F-23,31; M-18,18; MX-18,23,31. Mixture 2 genotypes are: F-24,28; M-18,24; MX 18,24,28. (The sample in the unlabeled lane is not part of the mixture study).*

heterozygous 23,31. In mixture two (right panel), the profiles overlap at the 24 allelic locus as a result of contribution by both individuals in the mixture sample. The female donor also contributes a dark 28 allele whereas the faint 18 allele is contributed only by the azoospermic donor. As would be expected, the bands of the major contributors (female) are preferentially amplified resulting in the more intense bands of the profile. Close evaluation of the actual gels aids in the interpretation of weak allelic profiles.

This PCR DNA analytic procedure offers a rapid, nonradioisotopic, sensitive and easily interpretable means of producing allelic profiles to aid the forensic scientist in casework. Furthermore, it illustrates that VNTR D1S80 allelic typing is quite suitable for the analysis of azoospermic semen. Allelic profiles found in all of the azoospermic semen samples matched the blood/salivary samples from the same individuals. This study provides a mechanism for recovering azoospermic seminal DNA in fraction one

of postcoital vaginal samples which can be compared with the semen donor's blood/saliva pattern. These data also suggest a method to render genotypic characterization of an alleged azoospermic sexual offender who may not have been previously typed because of the small amount of DNA recovered in evidentiary samples.

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