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TECHNICAL NOTE

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Analysis of Non-Suspect Samples Lacking Visually Identifiable Sperm Using a Y-STR 10-Plex

ABSTRACT: Y-STRs are valuable in the investigation of sexual assaults in which autosomal STR genotype interpretation is challenging. To detect male DNA from compromised sexual assault evidence, 45 non-suspect samples were differentially extracted and analyzed with 10 Y-STRs. These samples were positive for the presence of human seminal fluid, but were negative for spermatozoa by microscopic examination. Y-STR data were obtained in ~86.2% of the epithelial or sperm fractions. On samples yielding incomplete profiles, results were obtained on an average of 5 loci per sample. The inability to obtain results may be due to insufficient amplifiable male DNA, PCR inhibition, or unfounded accusations of sexual assault. This study indicates that it is possible to obtain a male STR profile even in the absence of visually identifiable spermatozoa. Furthermore, Y-STR loci should become components of CODIS if they are to be used in solving non-suspect sexual assaults.

KEYWORDS: forensic science, DNA typing, Y-chromosome, short tandem repeat, aspermic, sexual assault, DYS436, DYS439, DYS435, DYS19, DYS460, Y-GATA-H4, DYS391, DYS392, DYS438, DYS437

Y-chromosome short tandem repeats (Y-STRs) are important in forensic testing because of their ability to genetically identify the male component of a sample. Y-STR testing has played a critical role in sexual assault, immigration, genealogy, estate, and deficient paternity cases involving male offspring (1,2). In 1997, the U.S. Department of Justice estimated that 99% of the offenders in single-victim sexual assault incidents were male (3). Thus, the ability of forensic analysts to obtain male DNA profiles from sexual assault evidentiary samples can play a critical role in prosecuting these offenders.

In many sexual assault samples, such as vaginal swabs, the amount of female DNA overwhelms the quantity of male DNA present. Male DNA can appear in the epithelial fraction due to premature lysis of sperm or male epithelial cells present in the ejaculate (4). In these situations, the true genotype of the male suspect can be masked by the female victim's profile, making interpretation difficult. The interpretational problems can be compounded in the absence of reference sample(s), such as in non-suspect sexual assault casework. The absence of spermatozoa can also make analysis more challenging, and in some instances, aspermic samples are not even tested due to a potentially low ratio of male:female DNA. The present study utilized a Y-STR 10-plex (5) to determine the efficacy of Y-chromosome STRs in profiling male DNA from non-suspect sexual assault samples lacking visually identifiable spermatozoa. This Y-STR 10-plex was especially designed for amplification of compromised specimens in that all amplicons are less than 200 base pairs in length.

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Materials and Methods

DNA Samples

The male control DNA sample used was ATCC 45514 (American Type Culture Collection, Manassas, VA). Evidentiary samples ($n = 45$) included vaginal, anal, oral, and dried secretion swabs from Orchid Cellmark's non-suspect casework that were non-probative using standard nuclear STR analysis. DNA was isolated by a differential extraction procedure (6) to yield a total of 90 epithelial or sperm fractions. Three sperm fraction samples were not analyzed.

Presumptive and Confirmatory Testing

The evidentiary samples were subjected to three screening procedures unless otherwise noted: acid phosphatase (AP), p30, and microscopic examination for the presence of spermatozoa using Christmas Tree stain (7). Samples used in the present study were positive for both AP and p30, but were negative for the visual identification of sperm.

A snippet of each swab was reserved for AP testing, and the remainder of the swab was placed into a 1.5 mL microcentrifuge tube containing 750 μL of 1x AccuGENE® phosphate buffered saline (PBS) (BioWhittaker Molecular Applications, Rockland, ME). AP testing was performed according to the manufacturer's protocol using the AP Spot Test (Seri, Richmond, CA). Following an overnight incubation in PBS, the evidentiary swabs were placed into a spin basket and centrifuged for 5 minutes at 14,000 rpm. Approximately 700 μL of supernatant were removed and retained. Three microliters of the remaining PBS/cell mixture were placed on a microscope slide and allowed to dry. Following fixation with SprayCyte® (BD Biosciences, San Jose, CA), the cells were stained by the Christmas Tree method (7) and subsequently microscopically

examined. If spermatozoa were not observed, a p30 test was performed according to the manufacturer's protocol (Abacus Diagnostics, West Hills, CA).

Polymerase Chain Reaction (PCR) and Capillary Electrophoresis (CE)

Ten Y-chromosome microsatellites were amplified for 32 cycles in one PCR multiplex reaction as previously described by Johnson, et al. (5). Ten of the sperm fractions were amplified for 28 cycles using otherwise similar amplification conditions. The following loci were examined: DYS436, DYS439, DYS435, DYS19, DYS460, Y-GATA-H4 (H4), DYS391, DYS392, DYS438, and DYS437. Due to the limited amount of sample, the extracts were not quantitated prior to amplification. The volume of genomic DNA added to each reaction was 10 μL. PCR amplicons were subjected to capillary electrophoresis on the ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA) as previously described by Johnson et al. (5).

Results and Discussion

Table 1 (A–D) provides the results from the vaginal, anal, oral, and dried secretion specimens. Table 2 lists the results from the collective group of samples. Results are listed in terms of the type of profile obtained: full, full/partial, partial, and no results. In the present study, a full profile is defined as 10 Y-STR loci that produced results. A full/partial profile consists of 8–9 Y-STR loci that produced results, and a partial profile consists of 1–7 Y-STR loci that produced results.

Overall, full or full/partial Y-STR profiles were obtained in 29.9% and 16.1% of samples tested, respectively (Table 2). Partial profiles were obtained in 40.2% of samples tested, and no results were seen in 13.8% of the tested fractions. Therefore, 86.2% of samples that were negative for the presence of visually identifiable spermatozoa were able to yield usable male STR data. Samples that yielded incomplete profiles exhibited an average of 5 loci per sample. Of the samples that yielded results, approximately 56% were from epithelial fractions and 44% were from sperm fractions.

TABLE 2—*Results from the collective group of samples (vaginal, anal, oral, and dried secretion swabs). The results are given based on the type of profile obtained. The total percentage of fractions giving a result is listed in the "Percentage" column. The breakdown of this total percentage is listed in the "# EF" and "# SF" columns. These columns represent the number of each fraction that yielded a profile.*

Profiles	Percentage	# EF	#SF	
Full	29.9%	17	9	Total % swab fractions yielding
Full/partial	16.1%	6	8	data from 45 swabs: 86.2%
Partial	40.2%	19	16	(56% EF, 44% SF)
No results	13.8%	3	9	

The anal and dried secretion swabs produced the highest percentages of full profiles, at 44.4% and 50%, respectively, whereas the vaginal and oral swabs yielded 24.6–25% full profiles. The decreased number of full profiles observed in vaginal and oral samples may be due to the victim's showering, urinating, mouth rinsing, or tooth brushing following the sexual assault, thereby decreasing the quantity of male DNA present.

Numerous samples failed to yield data at one or more loci, which is likely caused by insufficient quantities of male DNA. However, a correlation between signal loss and PCR product size did not exist. Some swab samples exhibited more than one allele per locus. These results may be due to multiple assailants or an assailant and a consensual sexual partner contributing male DNA to the sample. Due to low copy number samples in many instances, these mixtures should be interpreted with caution since the possibility of allele drop-in exists.

TABLE 1—*Results from vaginal (A), anal (B), oral (C), and dried secretion (D) swabs from sexual assault kits. The results are given based on the type of profile obtained. The total percentage of fractions giving a result is listed in the "Percentage" column. The breakdown of this total percentage is listed in the "# EF" and "# SF" columns. These columns represent the number of each fraction that yielded a profile.*

A. Profiles	Percentage	# EF	# SF*	
Full	24.6%	10	4	Total % vaginal swab fractions
Full/partial	14.0%	5	3	yielding data from 30 swabs:
Partial	42.1%	12	11	78.9% (60% EF, 40% SF)
No results	19.3%	3	9	
* 3 SF samples were not analyzed				

B. Profiles	Percentage	# EF	# SF	
Full	44.4%	5	3	Total % anal swab fractions
Full/partial	16.7%	0	3	yielding data from 9 swabs:
Partial	38.9%	4	3	100% (50% EF, 50% SF)
No results	0%	0	0	

C. Profiles	Percentage	# EF	# SF	
Full	25.0%	1	1	Total % oral swab fractions
Full/partial	25.0%	1	1	yielding data from 4 swabs:
Partial	50.0%	2	2	100% (50% EF, 50% SF)
No results	0%	0	0	

D. Profiles	Percentage	# EF	# SF	
Full	50.0%	1	1	Total % dried secretion swab fractions
Full/partial	25.0%	0	1	yielding data from 2 swabs: 100%
Partial	25.0%	1	0	(50% EF, 50% SF)
No results	0%	0	0	

Several possibilities may explain the inability to obtain Y-STR data on approximately 1 in 7 of the samples. Inhibitors that quench the amplification reaction may have been present. Moreover, there may have been an insufficient quantity of amplifiable male DNA present in the specimens, or the DNA may have progressed to a state of degradation that made STR analysis unfeasible. Usage of a male-specific quantitation system such as Quantifiler Y (Applied Biosystems) will now allow analysts to determine if inhibitors and/or amplifiable male DNA are present in a sample. Lastly, some number of sexual assault claims are unfounded, and therefore, a profile may not have been observed because the alleged sexual assault may not have occurred, or there may have been non-penile penetration (by a foreign object, for example).

Epithelial cell DNA present in the seminal plasma of a vasectomized male can explain the Y-STR profiles obtained from the epithelial fractions (8). However, given the non-suspect samples were from sexual assaults that occurred approximately 6-12 years prior to Y-STR analysis, the more probable explanation involves the lysis of the sperm present on the swabs. The results of the AP, p30, and microscopic examination for spermatozoa support this hypothesis.

The ability to detect male DNA in the sperm fraction of a sample in which no visually identifiable sperm were observed can be explained in several ways. First, it is possible that during the differential extraction procedure there was insufficient washing of the sperm fraction, which resulted in a carryover of male epithelial cell DNA into that fraction. Secondly, microscopic examination for spermatozoa is a subjective process, and it is possible that spermatozoa were overlooked when viewing the slides. Lastly, in specimens that contained few sperm, there may have been poor sampling when making the slide, resulting in a negative identification for spermatozoa.

The results of the present study suggest that a visual lack of spermatozoa is insufficient to definitively determine whether or not an assault occurred. Furthermore, the presence of spermatozoa is not necessary for the successful genetic profiling of a male offender using Y-STR analysis.

This principle holds true when applied to other forensic casework. For example, evidence was submitted in a sexual assault case in which an adoptive father, who was vasectomized, allegedly molested a female child. Numerous pairs of panties were submitted for Y-STR testing, all of which were negative for sperm upon microscopic examination, yet p30 positive. Several of the tested items yielded male DNA in the epithelial fraction, all of which were consistent with the suspect. In another case, an adult female was sexually assaulted while unconscious due to excessive alcohol consumption. When she awoke, she found that her pants and tampon had been removed. She inserted a new tampon, which was later submitted for DNA testing. The tampon tested positive for AP and p30, but was negative for the presence of spermatozoa. Upon Y-STR testing, an 8-locus profile consistent with the suspect was obtained. These cases again illustrate the idea that visually identifiable sperm are not necessary for successfully obtaining a Y-STR profile.

Conclusion

In non-suspect cases in which autosomal testing yields a genotype, the profile is uploaded into the national convicted offender DNA profile database, CODIS, to search for a possible repeat offender. As yet, no such database exists for Y-STR haplotypes. To increase the usefulness of Y-chromosome data in sexual assault cases, the development of a national Y-STR database is essential. The first step of this process, deciding upon a set of core Y-STR markers to examine (the U.S. Y-STR haplotype), has already been accomplished (9). The present study utilized a Y-STR 10-plex that contains some, but not all, of these U.S. haplotype markers. A similar study using a commercially available kit such as Applied Biosystems' Yfiler or Promega's PowerPlex Y kit may also be performed to demonstrate that comparable results may be obtained, albeit with a different amplification multiplex. Standardization of Y-chromosome loci can allow laboratories to compile Y-STR haplotypes into a common, larger database, which will, in turn, increase the statistical power of the Y-STR data. However, disadvantages to the development of a national Y-STR database also exist. It is possible that a search could result in numerous matches between a piece of evidence and known Y-STR profiles in the database. Several database "hits" may needlessly raise doubt as to a suspect's involvement in a crime, as well as add to law enforcement's workload by increasing the number of leads that must be investigated. However, allowing for the simultaneous search of both a Y-STR profile and partial autosomal STR profile may reduce the number of investigative leads and provide valuable information.

The tested samples described here were previously excluded from autosomal STR testing based on presumptive testing, p30 results, and their lack of visually identifiable spermatozoa, but this served as the basis for sample selection in the present Y-STR study. As this paper demonstrates, Y-STR data were obtained from approximately 86% of the tested fractions. Although a Y-STR haplotype cannot uniquely identify an individual, it can serve to corroborate the victim's story or assess the validity of her statement. Based on this study, valuable DNA results may still be obtained from samples in which sperm are not visually identified. Therefore, one should consider performing autosomal and/or Y-chromosome STR testing on these types of samples in an effort to genetically identify the male component of sexual assault evidence.

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