JOURNAL OF FORENSIC SCIENCES

J Forensic Sci, November 2009, Vol. 54, No. 6
doi: 10.1111/j.1556-4029.2009.01180.x
Available online at: interscience.wiley.com

TECHNICAL NOTE

Alex M. Garvin,[1] Ph.D.; Michel Bottinelli,[2] Ph.D.; Mauro Gola,[2] Ph.D.; Ario Conti,[3] Ph.D.; and Gianni Soldati,[2] Ph.D.

DNA Preparation from Sexual Assault Cases by Selective Degradation of Contaminating DNA from the Victim

ABSTRACT: The standard method to purify sperm DNA from vaginal swabs taken from rape victims is to selectively digest the victim's epithelial cells to solubilize the victim's DNA, and then separate the soluble DNA from the intact sperm by centrifugation. A different approach to removing the soluble victim's DNA is to selectively degrade it using a nuclease, DNase I. DNase I reduces the amount of soluble DNA by over 1000-fold, while having virtually no effect on the sperm DNA remaining in the sperm head and inaccessible to the enzyme. Nuclease inactivation and sperm lysis then yield a soluble, pure male DNA fraction. An aliquot of soluble DNA is removed prior to nuclease addition to provide the victim's fraction. Vaginal swabs taken at defined time points following consensual sex and taken from rape victims were processed using the nuclease method or the standard method and the nuclease method gave superior short tandem repeat profiles.

KEYWORDS: forensic science, sexual assault evidence, automation, selective degradation, epithelial cells, spermatozoa, DNA typing, PowerPlex 16

Forensics laboratories are required to obtain short tandem repeat (STR) profiles (1) of sperm DNA obtained from vaginal swabs taken from rape victims, and the isolation of relatively pure sperm DNA from a vaginal swab continues to be a process that is tedious and difficult to automate. The standard differential lysis method for processing sexual assault cases (2) relies on separation of intact sperm from the DNA of digested epithelial cells by centrifugation and careful removal of supernatant, a process that remains unchanged since it was first described in 1985, in spite of efforts to improve this process (3,4). These efforts assume that the contaminating victim's DNA must be physically separated from the sperm. However, an entirely different approach is theoretically possible, namely to destroy the unwanted victim's DNA by selective degradation. Addition of a degradative agent is inherently easier than a physical separation process and can require only a single pipetting step. For such a method to work, the degradative agent must be highly selective (i.e., it must not degrade the sperm DNA) and it must be compatible with other steps in the overall process of obtaining polymerase chain reaction (PCR) ready male and female fractions from a swab cutting.

It is demonstrated that DNase I is highly selective for degrading solubilized epithelial cell DNA while not degrading sperm DNA present in intact sperm heads, and that DNase I is active in a modified detergent/proteinase K (ProK) buffer used to efficiently elute sperm off the swab cutting, therefore no buffer change is required. An aliquot of soluble DNA is removed prior to nuclease addition to yield the female fraction, and a soluble male fraction is obtained by inactivating the nuclease with EDTA and simultaneously lysing the sperm with dithiothreitol (DTT). The entire process required to obtain a pure soluble male fraction and a pure soluble female fraction from a vaginal swab cutting requires only six pipetting steps and no centrifugation.

[1]Bureco Corporation, Kagenstrasse 17, CH-4153 Reinach, Switzerland.
[2]Molecular Diagnostic Laboratory, Division of Forensic Genetics, c/o Cardiocentro Ticino, Via Tesserete 48, CH-6900 Lugano, Switzerland.
[3]Laboratory of Forensic Chemistry and Toxicology, Institute of Chemistry and Toxicology, 6718 Olivone, Switzerland.

Received 15 Aug. 2008; and in revised form 24 Nov. 2008; accepted 29 Nov. 2008.

Materials and Methods

Nuclease Protocol

The nuclease reagents can be conveniently mixed into four solutions and aliquoted for single use for each of the four steps required to go from swab cutting to soluble and pure male and female fractions. They are Solution 1 (500 μL aliquots): 2% Triton X-100, 20 mM Tris pH 8.0, ProK 400 μg/mL, and 1 mM EDTA; Solution 2 (25 μL aliquots): 40% glycerol, 1 mM EDTA, and 18 U/μL DNase I; Solution 3 (30 μL aliquots) 125 mM $CaCl_2$ and 125 mM $MgCl_2$; Solution 4 (25 μL aliquots): 1 M DTT and 500 mM EDTA. The aliquots can be stored indefinitely at −20°C. All of the ingredients for the three solutions were purchased from Sigma (St. Louis, MO) with the catalog numbers listed as: 95284 100 mL diethylpyrocarbonate (DEPC)-treated water, T-2694 100 mL 1.0 M Tris base solution, T9284 100 mL Triton X-100 Ultra pure, 82456-5ML ProK, G6279 500 mL glycerol, 8475 100 mL 2 M $MgCl_2$, 21115 100 mL 1 M $CaCl_2$, D5025-150KU DNase I, 03690 100 mL EDTA, 5 M D9779, and 5 g DTT.

EDTA is present in the ProK solution to keep endogenous nucleases from acting on the victim's DNA fraction. Triton X-100 substitutes for sodium dodecyl sulfate (SDS; the detergent used in the standard protocol) because SDS inhibits DNase I. The DNase I in Solution 2 is inactive due to the presence of EDTA. The enzyme is activated by magnesium and calcium in Solution 3 and then inactivated in the same tube by a large excess of EDTA in Solution 4,

therefore the nuclease is only active when needed and poses little threat to DNA samples that might be contaminated by trace amounts of Solution 2.

Cell Samples Were Treated Using the Following Protocol

(1) Remove tube 1 from the freezer, thaw at 56°C for 2 min. Add sample to tube 1, vortex for 10 sec and place at 56°C. The sample can be epithelial cells in solution, fresh semen, a buccal swab cutting, or a vaginal swab cutting. For a swab cutting only add the outer layer of the swab that contains the cells, maximum 15 mg of swab cutting. (2) After 10 min incubation, remove 50 μL aliquot from the upper part of the liquid and place in a new tube. This is the female fraction. (3) Continue the incubation of tube 1 during 4 h at 56°C. (4) Remove tubes 2, 3, and 4 from the freezer. Set aside tubes 3 and 4 at room temperature to thaw. (5) Vortex tube 1 for 10 sec and then pipette 350 μL from tube 1 to tube 2. (6) Add 25 μL of tube 3 to tube 2, mix gently by inverting 10 times and incubate 56°C for 1 h. Do not vortex. Discard tubes 1 and 3. (7) After 1 h incubation, add 20 μL from tube 4 to tube 2, vortex for 10 sec, and incubate at 56°C for 5 min. Discard tube 4. Tube 2 is the male fraction.

The male and female fractions are now ready for purification and analysis. The following method uses Qiagen reagents from the Qiamp DNA Mini Kit (Qiagen, Valencia, CA). (1) Add an equal volume of Qiagen AL buffer to the male and female fractions. (2) Add an equal volume of ethanol to the male and female fractions. (3) Load Micro Column. (4) Wash with 500 μL Qiagen AW1 buffer. (5) Wash with 500 μL Qiagen AW2 buffer. (6) Elute with 20 μL 2x Qiagen AE buffer (EDTA level must be 1 mM or less). The buffers are also compatible with the Invitrogen Charge/Switch chemistry (Invitrogen Corporation, Carlsbad, CA).

Standard Selective Lysis Protocol

Control experiments using the standard selective lysis protocol (2) relied on a recently published version of this method that uses only commercially available reagents for the lysis and washing buffers, namely the Protocol for Isolation of Total DNA from Sexual Assault Cases, page 48 of the Qiagen Qiamp DNA Investigator Handbook. The final elution volume was 20 μL.

Differex System

Swabs obtained from the police were processed in parallel using the Differex System for sexual assault cases (Promega, Madison, WI) followed by DNA purification with the Qiagen Qiamp DNA Mini Kit. The protocol is that used by the Institute of Molecular Diagnostics in Lugano. Briefly, a swab cutting is digested with 380 μL of the Differex Digestion buffer and ProK for 2 h at 56°C and then spun simultaneously through a DNA IQ spin basket (Promega) and 100 μL of Differex Separation Solution; 400 μL of the supernatant is removed for the female fraction and the sperm/separation solution is washed twice with 500 μL distilled water, leaving in place the 100 μL of separation solution each time. The sperm pellet is then resuspended in 180 μL Qiagen ATL buffer and digested with ProK and DTT and then purified with the standard Qiagen Qiamp DNA Mini Kit protocol.

DNA Quantitation

DNA yields were determined by quantitative PCR using 18S primers (Eurogentec, Seraing, Belgium) and SYBR Green readout (Power SYBR Green; Applied Biosystems, Foster City, CA); 3 μL of a DNA solution was placed in 75 μL of 1x Power SYBR Green with 50 nM primers, and 25 μL reactions were run in triplicate on an Applied Biosystems 7500 Real Time PCR System using standard cycling parameters (50°C for 2 min, 95°C for 10 min, 40 cycles 95°C for 15 sec, 60°C for 1 min). A standard curve was generated for each run with standards from 10 ng to 1 pg at 1 log intervals.

STR Profiling

DNA samples were profiled using the PowerPlex 16 system from Promega; 500 pg of DNA was used as input when possible, and when less than this amount was available for analysis the entire DNA sample was used as template for amplification. The volume of added DNA was never more than 17 μL and the EDTA concentration in the DNA solution was 1 mM (2x Qiagen AE buffer). Amplification products were separated on an Applied Biosystems 310 Genetic Analyzer and the data were analyzed using Genescan software (Applied Biosystems). Allele scoring was performed by direct comparison of the published PowerPlex 16 allele ladder (Promega) and the PowerPlex 16 allele ladders run as controls with each set of STR profiles. The alleles seen in each sample were correlated to the alleles in the ladder by using the absolute sizes as determined by the Genescan software using the ILS600 size standard.

Cell Samples

Three buccal swabs from three different individuals were dried for 4 weeks at room temperature (unexposed to light). Cells from an entire swab were then eluted in 1 mL of Solution 1 (detergent and ProK) by rotating the swab for 1 min in the solution and then pressing the swab against the side of the tube to reduce the amount of liquid remaining on the swab. The solution was then incubated at 56°C for 4 h to digest the epithelial cells. The volume following elution was about 800 μL due to retention of some liquid by the swab. The samples were split in half to generate two sets of identical epithelial cell digests; 20 μL of Solution 2 (the nuclease) was added to one set and not the other, and the tubes were incubated at 56°C for 1 h; 20 μL of Solution 3 was then added to all six tubes and incubated for 5 min at 56°C.

Either 4000, 12,000, or 36,000 sperm from a 10% semen solution (having 3000 sperm/μL) was added to 1 mL of Solution 1 and processed in the same way as the buccal cells above.

Timed postcoital vaginal swabs following consensual sex and semen were obtained from healthy volunteers. Semen was diluted to 10% in phosphate-buffered saline and the sperm count of this stock solution was determined by hemocytometry. Five nonprobative vaginal swabs taken from rape victims were obtained from the Police Force of the Canton of Ticino, Switzerland. These swabs had no information (other than a number from 1 to 5) attached to them and had been at room temperature and unexposed to light from 2 to 10 years (depending on the swab) prior to processing. The STR profiles given in Table 1 have been altered to protect the privacy of the swab donor.

Vaginal swabs were cut in half lengthwise with a scalpel and half from each swab was randomly assigned for processing using either the nuclease protocol or the Differex protocol (for the swabs from rape victims) or the nuclease protocol and the Qiagen version of the selective lysis method (postcoital swabs following consensual sex).

TABLE 1—*STR profiles of five vaginal swabs taken from rape victims.*

Locus	Fraction	Swab 1		Swab 2		Swab 3		Swab 4		Swab 5	
CSFIPO	Female	11		12	13	12	13	11	12	13	14
	Std male	12	13	?		12	13	11	12	11	13
	Nuc male	12	13	11	13	12	13	12	13	11	13
FGA	Female	23	24	20		23	24	20	24	21	23
	Std male	19	25	?		?		20	24	18	22
	Nuc male	19	25	22	23	?		?		18	22
THO	Female	6	8	6	7	7	10	9	9.3	9	9.3
	Std male	6	7	?		?		9	9.3	6	7
	Nuc male	6	7	7	9	?		7	9.3	6	7
TPOX	Female	8	12	8		8	11	8	11	9	
	Std male	9	10	?		8	11	8	11	11	12
	Nuc male	9	10	8	10	8	11	11		11	12
VWA	Female	13	17	17		15	18	16	18	16	
	Std male	17	18	17		15	18	16	18	16	17
	Nuc male	17	18	17		15	18	?		16	17
D3S1358	Female	16	18	14	18	16	18	14	16	14	15
	Std male	16	17	?		?		14	16	16	18
	Nuc male	16	17	15	18	?		16		16	18
D5S818	Female	11	12	13	14	10	11	11		11	12
	Std male	10	11	?		?		11		11	12
	Nuc male	10	11	12		?		11	13	11	12
D7S820	Female	10	12	8	11	8	9	8	11	10	
	Std male	8	11	8	11	?		8	11	8	14
	Nuc male	8	11	8	11	?		9		8	14
D8S1179	Female	11	13	15		11	12	11	12	12	13
	Std male	13	14	?		?		11	12	12	15
	Nuc male	13	14	13	14	?		12	14	12	15
D13S317	Female	10	11	9	13	11	12	12	13	9	12
	Std male	11		?		11	12	12	13	8	12
	Nuc male	11		8	10	11	12	11	12	8	12
D16S539	Female	9	13	11	12	12	13	9	10	9	14
	Std male	10		?		?		9	10	11	14
	Nuc male	10		8	11	?		8	12	11	14
D18S51	Female	14	17	16		12	17	14	19	17	
	Std male	13	18	?		?		14	19	13	17
	Nuc male	13	18	12	13	?		12	14	13	17
D21S11	Female	28	30	28	30.2	27	28	30	33.2	30	32
	Std male	28	31.2	?		?		30	33.2	31	32.2
	Nuc male	28	31.2	29		?		28		31	32.2
Amelogenin	Female	X		X		X		X		X	
	Std male	X	Y	X		X		X		X	Y
	Nuc male	X	Y	X	Y	X		X	Y	X	Y

Results

Nuclease Treatment of Epithelial Cells or Sperm

Buccal cells from three individuals were digested with ProK, split in half, and treated either with or without the nuclease (see Materials and Methods). Identical experiments were performed with 4000, 12,000, or 36,000 sperm from a 10% semen solution. The DNA was purified and quantitated and the yields are shown in Table 2. Nuclease treatment reduces the amount of DNA from the buccal cell samples by over 1000-fold to sub-nanogram levels, while having only a slight effect on the semen samples. Semen is known to contain epithelial cells and other nonsperm cell types that are susceptible to ProK digestion, and the slight reduction in DNA levels is presumably due to the degradation of the nonsperm DNA present in semen.

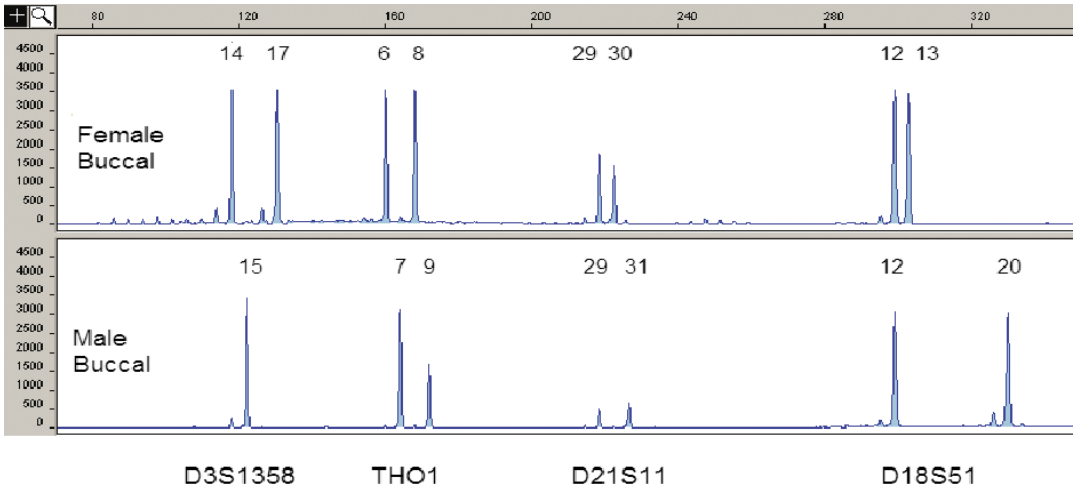
TABLE 2—*DNA yields from epithelial cells and sperm, with and without nuclease treatment.*

Cells	DNase I	Yield (pg)	Fold Reduction
Buccal swab 1	–	340,000	1133
	+	300	
Buccal swab 2	–	140,000	4666
	+	30	
Buccal swab 3	–	800,000	2857
	+	280	
4000 Sperm	–	6400	1.39
	+	4600	
12,000 Sperm	–	16,800	1.08
	+	15,600	
36,000 Sperm	–	79,460	1.11
	+	71,220	

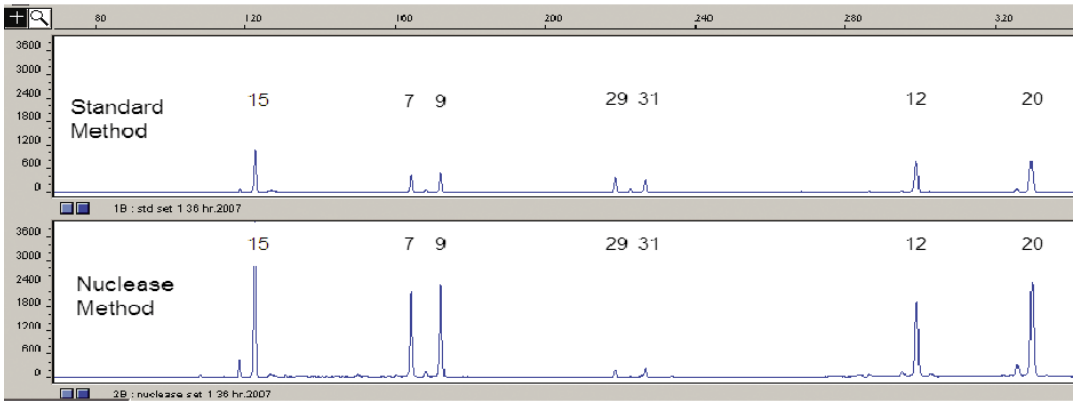
Processing Postcoital Vaginal Swabs Following Consensual Sex

A series of postcoital vaginal swabs was obtained from a volunteer couple and used to compare the standard selective lysis method directly with the nuclease method. Swabs were cut in half lengthwise and swab halves were randomly assigned for processing by either method. The standard method is essentially that of Gill et al. (2) with three washing steps using Qiagen reagents and the published Qiagen protocol. As expected, swabs taken soon after sex gave clear male profiles with both methods, because such swabs have large numbers of sperm. At 36 h, both methods give unambiguous male profiles that match the male buccal control, as shown in Fig. 1*b*. A number of swabs taken between 1 and 30 h also gave clear male profiles with both methods (data not shown). With

a) Buccal swab controls



b) Male fractions from vaginal swab at 36 hours



c) Male fractions from vaginal swab at 61 hours

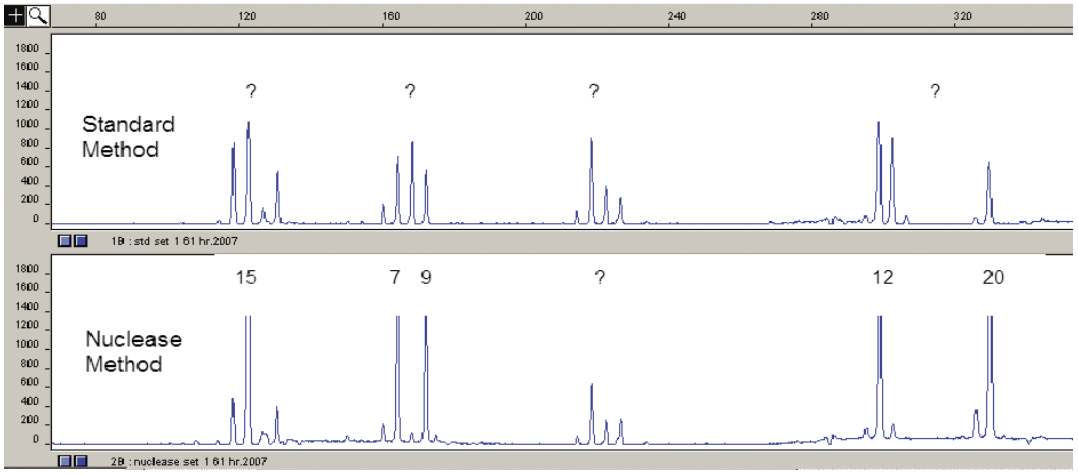
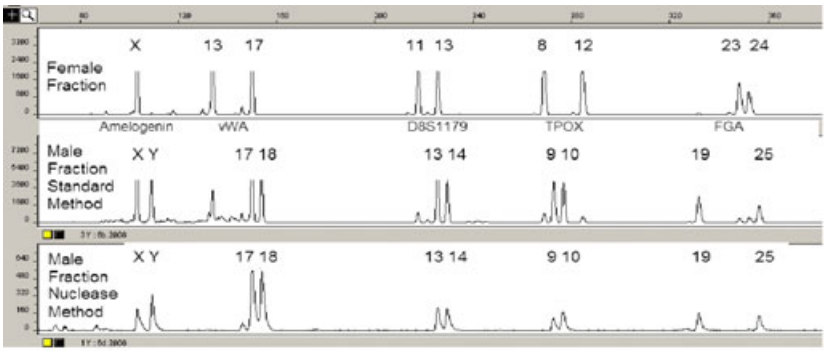
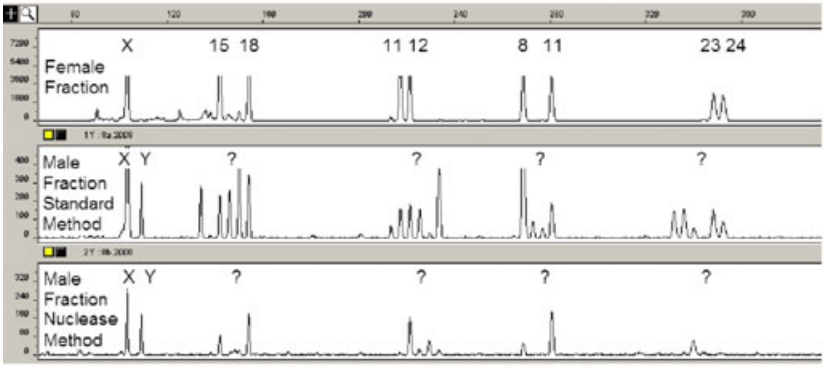


FIG. 1—*Four STR loci (D3S1358, THO1, D21S11, and D18S51) from the PowerPlex 16 kit for buccal swabs and male fractions from postcoital vaginal swabs. (a) Buccal swab DNA profiles show that the male and female have different profiles at all four loci. (b) Male fractions obtained from a 36 h postcoital swab give a correct male profile with both the standard selective lysis protocol and the nuclease protocol. (c) Swab cuttings from 61 h postcoital swab yield a correct male profile for three of the four loci with DNA prepared with the nuclease method, while none of the loci give the correct male profile using DNA prepared using the standard method.*

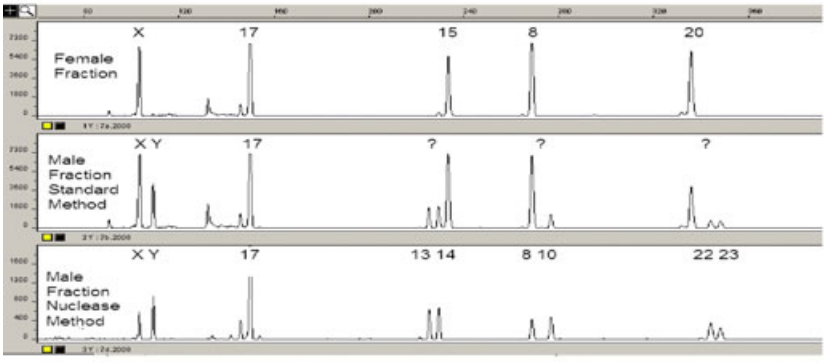
a) Swab 1



b) Swab 3



c) Swab 2



d) Swab 4

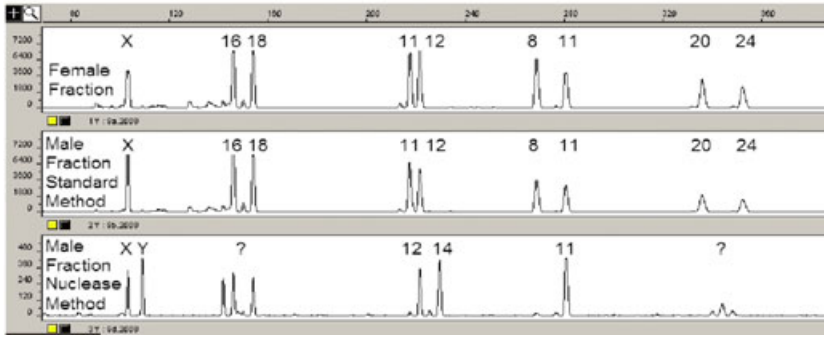


FIG. 2—*Amelogenin plus four STR loci (vWA, D8S1179, TPOX, and FGA) for a swab where both the Differex and the nuclease methods gave good male profiles (swab 1), where both methods gave poor profiles (swab 3), and where the nuclease method gave superior profiles (swabs 2 and 4).*

increasing time, however, the number of sperm collected on the swab is expected to drop, and at some point the number of sperm will be so low that no method can give a clear male autosomal STR profile. This was observed with swabs taken at 70–100 h (data not shown). At 61 h (Fig. 2*c*), however, the nuclease method gave superior results and the correct male loci are easily determined at three of the four loci shown, while the standard method yielded DNA that was contaminated with female DNA to the point where a clear determination of the male profile was not possible at any of the loci.

Processing Vaginal Swabs from Rape Victims

The Cantonal Police of Ticino, Switzerland, provided five archived swabs from rape victims for comparative testing. The Diagnostics Laboratory in Lugano, Switzerland, uses a modified and commercially available version of the standard method (Differex), which was used in comparison to the nuclease method. Male and female DNA fractions were profiled with PowerPlex 16 and the STR profiles for the 13 core STR loci used by the FBI are given in Table 1 for the five swabs. A question mark at a particular locus in Table 1 indicates that no genotype was obtained. Two swabs (swabs 1 and 5) gave good male profiles with both methods presumably because the ratio of sperm to epithelial cells in the starting material was high. One swab (swab 3) did not give a useful male profile with either method and most likely had very few sperm.

The remaining two swabs (swabs 2 and 4) gave superior results with the nuclease method. Differex DNA from swab 2 gave a mixture, while Differex DNA from swab 4 gave an entirely female profile. However, the nuclease method yielded male DNA from these swabs that gave a clear male profile for all 13 loci for swab 2, and 11 of the 13 loci for swab 4. The amelogenin locus and four other loci (vWA, D8S1179, TPOX, and FGA) are shown for representative swabs in Fig. 2.

Discussion

The selective lysis process is tedious, time consuming, and difficult to automate, and a number of approaches have been attempted to circumvent this method. For example, Y chromosome polymorphic markers can be amplified from unfractionated swab DNA (5–10). However, this approach has the following disadvantages: the data provided cannot be used to probe the autosomal STR profiles in the FBI CODIS database, it won't work when the rape victim is male, and most importantly, males of the same paternal lineage usually have identical Y chromosome STR patterns due to the lack of independent segregation and homologous recombination of Y chromosomes during meiosis. As some paternal lineages contain many thousands of males with the same Y chromosome STR pattern, Y chromosome profiles have limited utility compared with autosomal profiles as they do not provide the identity of the rapist.

Another approach toward avoiding selective lysis is to physically separate sperm from intact epithelial cells. This has been carried out by flow cytometry (11); however, this technique is unlikely to be applied to casework due to the expense of cell sorters and the difficulty of operating them. Attempts have also been made to use anti-sperm antibody-coated magnetic beads. Epitope stability, however, was a problem with this approach when applied to casework because detergents such as Sarkosyl, SDS, or Triton X-100 are required to efficiently elute sperm from the swabs and these detergents destroy the epitopes recognized by the anti-sperm antibodies. Sperm can also be physically separated from the much larger intact epithelial cells by size using a 10-μm filter (12) or from digested epithelial cells by collection on 2-μm filter (13). However, these filtration methods still require centrifugation, and do not provide male fraction DNA from postcoital vaginal swabs that is as good or better than that provided by the standard method. Laser dissection of sperm from a slide has also been proposed (14–16), but this method is low throughput and will most likely not be adopted for routine processing of sexual assault cases.

The Differex method used in this study and a new version of Differex that attempts some degree of automation (see the Automated Differex System at http://www.Promega.com) both require a number of manual steps to process a swab cutting to soluble and pure male and female fractions, and manual centrifugation steps are required for both processes. The nuclease method requires only six pipetting steps, controlled incubations, and shaking (or vortexing), which are processes that existing robotic work stations can easily do. The output for the nuclease method is a male fraction and a female fraction of soluble and pure DNA ready for further purification using any number of well-established DNA automated sample preparation methods, such as guanidinium/paramagnetic silica beads.

Each of the 14 male fraction samples provided enough DNA to get STR profiles, so DNA yield was not the issue, although male DNA yields did vary substantially between swabs (data not shown). The problem with some male fractions is not the amount of DNA per se, but rather the amount of contaminating female DNA relative to the amount male DNA. All vaginal swabs contain many thousands of the victim's epithelial cells and thus large amounts of the victim's DNA. Both the standard selective lysis method, Differex, and the nuclease method are efficient in removing the victim's DNA while leaving the sperm DNA intact, although these experiments show that the nuclease method provides a superior male fraction. This is most likely due to the fact that some sperm are inevitably lost during the separation and washing steps required for the first two methods, while the nuclease method does not require washing or separation steps.

References

1. Butler JM. Short tandem repeat typing technologies used in human identity testing. BioTechniques 2007;43:ii–v.
2. Gill P, Jeffreys AJ, Werrett DJ. Forensic application of DNA ''fingerprints''. Nature 1985;318:577–9.
3. Yoshida K, Sekiguchi K, Mizuno N, Kasai K, Sakai I, Sato H, et al. The modified method of two-step differential extraction of sperm and vaginal epithelial cell DNA from vaginal fluid mixed with semen. Forensic Sci Int 1995;72:25–33.
4. Wiegand P, Schurenkamp M, Schutte U. DNA extraction from mixtures of body fluid using mild preferential lysis. Int J Legal Med 1992;104: 359–60.
5. Dekairelle AF, Hoste B. Application of a Y-STR-pentaplex PCR (DYS19, DYS389I and II, DYS390 and DYS393) to sexual assault cases. Forensic Sci Int 2001;118:122–5.
6. Delfin FC, Madrid BJ, Tan MP, De Ungria MC. Y-STR analysis for detection and objective confirmation of child sexual abuse. Int J Legal Med 2005;119:158–63.
7. Hall A, Ballantyne J. Novel Y-STR typing strategies reveal the genetic profile of the semen donor in extended interval post-coital cervicovaginal samples. Forensic Sci Int 2003;136:58–72.
8. Mayntz-Press KA, Sims LM, Hall A, Ballantyne J. Y-STR profiling in extended interval (> or = 3 days) postcoital cervicovaginal samples. J Forensic Sci 2008;53:342–8.
9. Sinha SK, Budowle B, Chakraborty R, Paunovic A, Guidry RD, Larsen C, et al. Utility of the Y-STR typing systems Y-PLEX 6 and Y-PLEX 5 in forensic casework and 11 Y-STR haplotype database for three major population groups in the United States. J Forensic Sci 2004;49:691–700.
10. Tsuji A, Ishiko A, Ikeda N, Yamaguchi H. Personal identification using Y-chromosomal short tandem repeats from bodily fluids mixed with semen. Am J Forensic Med Pathol 2001;22:288–91.
11. Schoell WM, Klintschar M, Mirhashemi R, Pertl B. Separation of sperm and vaginal cells with flow cytometry for DNA typing after sexual assault. Obstet Gynecol 1999;94:623–7.

12. Chen J, Kobilinsky L, Wolosin D, Shaler R, Baum H. A physical method for separating spermatozoa from epithelial cells in sexual assault evidence. J Forensic Sci 1998;43:114–8.
13. Garvin AM. Filtration based DNA preparation for sexual assault cases. J Forensic Sci 2003;48:1084–7.
14. Anslinger K, Mack B, Bayer B, Rolf B, Eisenmenger W. Digoxigenin labeling and laser capture microdissection of male cells. Int J Legal Med 2005;119:374–7.
15. Di Martino D, Giuffre G, Staiti N, Simone A, Le Donne M, Saravo L. Single sperm cell isolation by laser microdissection. Forensic Sci Int 2004;146:S151–3.
16. Sanders CT, Sanchez N, Ballantyne J, Peterson DA. Laser microdissection separation of pure spermatozoa from epithelial cells for short tandem repeat analysis. J Forensic Sci 2006;51:748–57.

Additional information and reprint requests:
Alex M. Garvin, Ph.D.
Bureco Corporation
17 Kagenstrasse
CH-4153 Reinach
Switzerland
E-mail: Alex.Garvin@Bureco.com