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Y-STR Profiling in Extended Interval (≥3 days) Postcoital Cervicovaginal Samples*

ABSTRACT: Depending upon specific situations, some victims of sexual assault provide vaginal samples more than 36–48 h after the incident. We have tested the ability of commercial and in-house Y-STR systems to provide DNA profiles from extended interval (≥3 days) postcoital samples. The commercial Y-STR systems tested included the AmpFℓSTR® Yfiler™ (Applied Biosystems), PowerPlex® Y (Promega) and Y-PLEX™ 12 (Reliagene) products whereas the in-house systems comprised Multiplex I (MPI) and Multiplex B (MPB). Three donor couples were recruited for the study. Postcoital cervicovaginal swabs (x2) were recovered by each of the three females at specified intervals after sexual intercourse (3–7 days). Each time point sample was collected after a separate act of sexual intercourse and was preceded by a 7-day abstention period. As a negative control, a precoital swab was also recovered prior to coitus for each sampling and only data from postcoital samples that demonstrated a lack of male DNA in the associated precoital sample was used. A number of DNA profile enhancement strategies were employed including sampling by cervical brushing, nondifferential DNA extraction methodology, and post-PCR purification. Full Y-STR profiles from cervicovaginal samples recovered 3–4 days after intercourse were routinely obtained. Profiles were also obtainable 5–6 days postcoitus although by this stage partial profiles rather than full profiles were a more likely outcome. The DNA profiles from the sperm fraction of a differential lysis were superior to that obtained when a nondifferential method was employed in that the allelic signal intensities were generally higher and more balanced and exhibited less baseline noise. The incorporation of a simple post-PCR purification process significantly increased the ability to obtain Y-STR profiles, particularly from 5- to 6-day postcoital samples. Remarkably an 8 locus Y-STR profile was obtained from a 7-day postcoital sample, which is approaching the reported time limit for sperm detection in the cervix.

KEYWORDS: forensic science, Y-STRs, multiplex DNA typing systems, AmpFℓSTR® Yfiler™, PowerPlex® Y, Y-PLEX™ 12, MPI, MPB, extended interval postcoital cervicovaginal samples, post-PCR purification, MinElute™

Depending upon specific situations, some victims of sexual assault provide vaginal samples more than 36–48 h after the incident. In these cases, the ability to obtain an autosomal STR profile of the semen donor from the living victim diminishes rapidly as the postcoital interval is extended (1). Although it may be possible, though unlikely in most instances, to obtain an autosomal STR profile of the semen donor from vaginal samples taken 24–36 h after intercourse, it is normally not possible to do so when the postcoital interval exceeds 48 h (1). However, for many of these cases, the failure to detect the genetic signature of the male donor is not due to the absence of male cells. Classical forensic serology studies have shown consistently that spermatozoa, albeit few in number, persist in the vaginal canal 3 days after intercourse and even longer (2–6). Moreover the reproductive biology literature is replete with reports demonstrating the presence of several spermatozoa in the human cervix up 7–10 days postcoitus, which is consistent with the concept of the cervix as a sperm repository prior to fertilization (4,7–10). The question thus arises as to why the sensitive methods of forensic DNA analysis routinely fail to detect these male cells.

The reasons for the inability to detect the genetic profile of the male donor in extended interval postcoital vaginal samples can be attributed to a combination of sperm loss or lysis and the technological limitations of the DNA typing systems employed (1). Sperm loss after intercourse is due to vaginal lavage and drainage, menstruation, and the normal intra-cervicovaginal sperm degradative changes that occur over time. As a result of the latter process the few remaining sperm are expected to be in a structurally fragile state due to a somewhat damaged outer membrane. Loss can also occur during the multiple manipulations required of the differential lysis process used to separate the sperm from the nonsperm DNA fractions within the laboratory. The overwhelming majority of the DNA components in the nonsperm fraction comprise that from the vaginal epithelial cells from the victim. In addition to sperm loss, premature lysis of the few remaining fragile sperm during the differential extraction process will result in male DNA becoming admixed with female DNA.

The technological impediments to typing success with extended interval postcoital samples pertain to the low copy numbers of DNA templates present as well as the detection sensitivity of the autosomal STR systems employed. Standard protocols permit the detection of as little as 50–100 pg of DNA (11–15), which is roughly equivalent to 17–33 haploid (sperm) cells, but the sperm fraction in such samples may contain fewer cells (<10) and hence may be below the analytical detection limit of the system. Moreover, DNA from sperm cells that have prematurely lysed into the nonsperm (or female epithelial cells) fraction may be undetectable due to the kinetics of the PCR process itself. In those nonsperm

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cell fractions containing prematurely lysed sperm in which the female/male DNA ratio is sufficiently large the minor component (male) would not be detectable, since the major contributor (female) would out-compete for, and titrate out, the critical PCR reagents required for male DNA amplification (16–19). An obvious solution to this problem would be to substitute Y chromosome markers for the standard set of autosomal markers currently employed. The demonstrated efficacy and high sensitivity of Y-STRs for discerning the genetic profile of the male donor in admixtures of body fluids has resulted in the increasing use of these markers in sexual assault cases (16–22).

Previously we have developed specific sub-sets of the core Y-STR loci (designated MPA and MPB) to detect the male profile in samples recovered 4 days after intercourse (1). Thus it becomes possible to derive an 11–19 locus Y-STR profile of the semen donor in cervicovaginal samples recovered 2–4 days after intercourse (1). In the present work, we have sought to consolidate and confirm these previous findings and further extend, if possible, the postcoital time limit for which a male profile can be obtained from intimate samples. Specifically we tested the ability of commonly used commercial Y-STR systems, namely the AmpFℓSTR® Yfiler™ (Applied Biosystems) (23), PowerPlex® Y (Promega) (24), and Y-PLEX™ 12 (Reliagene) (25) products, to provide DNA profiles from extended interval postcoital samples. In addition a number of potential DNA profile enhancement strategies were employed including sampling by cervical brushing, nondifferential DNA extraction methodology, and post-PCR purification.

Materials and Methods

Sample and Sample Preparation

Two postcoital swabs were obtained by each of three female volunteers who recovered the samples after separate acts of sexual intercourse at various time points using sterile cotton tipped applicators (Puritan, Guilford, MA). The volunteers were instructed to take the cervicovaginal swabs by brushing the cervix multiple times for at least 30 sec at each specific postcoital time interval. To insure that the postcoital swabs were void of any previous male DNA a precoital cervicovaginal swab was also obtained before coitus commenced but after an abstinence period of 7 days. The time points collected were: 72, 96, 120, 144, and 168 h.

DNA Isolation and Purification

DNA was extracted from the samples using both a standard organic extraction and differential organic extraction protocol (26). Whole swab tips were placed in a Spin-Ease tube (Gibco-BRL, Grand Island, NY) and incubated overnight at 56°C in 400 μL DNA extraction buffer (100 mM NaCl, 10 mM Tris-HCl, pH 8.0, 25 mM EDTA, 0.5% SDS, 0.1 mg/mL Proteinase K). The swab tip was removed from the tube and placed into a Spin-Ease basket and placed back into its original tube and centrifuged at 16,100×*g* for 5 min to ensure all absorbed fluid was removed from the swab tip. The extract was purified by adding 400 μL of 25:24:1 phenol/chloroform/isoamyl alcohol (Fisher Scientific, Norcross, GA) to the Spin-Ease tube. The upper organic layer was removed and added to a Microcon concentrator (Millipore, Bedford, MA) for purification according to the manufacturer's instructions. Samples (*c.* 25 μL total extract) were stored in TE^{-4} (10 mM Tris-HCl, 0.1 mM EDTA, pH 7.5) and kept at 4°C until analysis. Three hundred nanograms of DNA of this ''nondifferential'' isolate was used for amplification. In some experiments, sperm and nonsperm cells were separated using a standard differential lysis protocol, with minor modifications (17). Postcoital cervicovaginal swabs were incubated at 37°C in 400 μL of DNA extraction buffer (100 mM NaCl, 10 mM Tris-HCl, pH 8.0, 25 mM EDTA, 0.5% SDS, 0.1 mg/mL Proteinase K). The swab tip was removed from the tube and placed into a Spin-Ease basket and placed back into its original tube and centrifuged at 16,100 *g* for 5 min to ensure all absorbed fluid was removed from the swab tip. The resulting supernatant, containing the nonsperm DNA fraction, was removed into a separate tube for further analysis. The sperm pellet was resuspended in 400 μL of DNA extraction buffer, and 0.1 mg/mL Proteinase K and 40 μL of 0.39 M DTT and incubated overnight at 56°C. DNA from both the sperm and nonsperm fractions was isolated and purified using the phenol: chloroform method described above. If sufficient quantity was isolated, 1 ng of the sperm fraction DNA was used for amplification. For some samples (i.e., those recovered 6–7 days postcoitus) <1 ng total DNA was isolated, in which case 5 μL of the *c.* 25 μL extract was used for amplification.

DNA Quantitation

DNA was quantitated using the Quantifiler™ Human DNA Quantification Kit and Quantifiler™ Human Male DNA Quantification Kit (Applied BioSystems, Foster City, CA) (27) in accordance with the manufacturer's instructions.

Standard PCR Conditions

The amplification, separation, and data analysis of the MPI, MPB, Yfiler™, PowerPlex® Y, and Y-PLEX™ 12 multiplex Y-STR systems were carried out as previously described (28). The Y-PLEX™ 12 system (Reliagene Technologies, Inc., New Orleans, LA) is no longer available as a commercial product.

Post-PCR Clean Up

In some experiments, amplified product from commercial Y-STR kits (AmpFℓSTR® Y-Filer™ Kit [Applied Biosystems] and PowerPlex® – Y System [Promega Corporation, Madison, WI]) was purified by the MinElute™ PCR purification spin column (Qiagen Sciences, Germantown, MD) as previously described (29).

Results

Three donor couples were recruited for the study. Postcoital cervicovaginal swabs (x2) were recovered by each of the three females at specified intervals after sexual intercourse (3–7 days). Each time point sample (72, 96, 120, 144, and 168 h) was collected after a separate act of sexual intercourse and was preceded by a 7-day abstention period. As a negative control, a precoital swab was also recovered prior to coitus for each sampling and only data from postcoital samples that demonstrated a lack of male DNA in the associated precoital sample was used. Two different DNA extraction procedures were employed (1,17,26,30), after which the isolated DNA was quantified by human- and male-specific real time PCR assays that detect the human telomerase reverse transcriptase and Y-chromosome SRY genes respectively. One whole postcoital swab was used for each of the two DNA extraction procedures. The first DNA isolation method comprised a standard differential lysis procedure in which 1 ng of the sperm fraction (or 5 μL of the extract if there was <1 ng male DNA isolated) was used for Y-STR analysis (1,26). Secondly, a

nondifferential extraction method was used, from which 300 ng of DNA was used for Y-STR amplification.

Although the emphasis of this study was the extent to which different commercial Y-STR systems were capable of obtaining male DNA profiles from varying postcoital intervals, we also tested previously well characterized in-house Y-STR systems as performance yardsticks. The commercial systems evaluated were the AmpFℓSTR® Yfiler™ PCR Amplification Kit (Applied Biosystems) (23), the PowerPlex® Y System (Promega) (24), and Y-PLEX™ 12 (Reliagene) (25). The amplification conditions employed were those recommended by the manufacturers. MPI is a "first generation" multiplex system containing nine loci (1,17), whereas MPB comprises a set of five loci that can detect the male donor profile in cervicovaginal samples recovered 4 days after intercourse (1).

DNA Isolation by Differential Lysis

The typing results of the postcoital DNA samples subjected to a differential lysis protocol are summarized in Table 1. Complete Y-STR profiles of the male donors were obtained 3–4 days postcoitus with all Y-STR systems tested (Fig. 1), except for Y-Plex™ 12, which gave a full profile with one of the donor couples (#1) and no profile with the other couple tested (#2) after 4 days. Examples of electrophereograms obtained for the 3-day postcoital samples are provided in Fig. 1.

Notably, full or partial Y-STR profiles were obtained from the majority of the 5-day postcoital samples (Fig. 2), the only exception being the Y-Plex™ 12 system with one of the donor couples (#1).

Remarkably, Y-STR profiles from the two commonly used commercial systems (Yfiler™ and PowerPlex® Y) were obtained from postcoital samples recovered 6 days after intercourse. These profiles were partial in nature and the relative typing success appeared to be sample dependent: 11 Y-STR loci (couple #1), 6–9 loci (couple #2), and 1–3 loci (couple #3).

Six days appears to be the limit for obtaining Y-STR profiles using standard methodology since no profiles were obtained from the 7-day postcoital samples, with the possible exception of one locus from one sample using one of the commercial kits (Table 1).

TABLE 1—*Y-STR typing results of postcoital samples.*

Y-STR System	Donor Couple	Precoital Samples	Postcoital Interval (Days)				
			3	4	5	6	7
Y-filer	1	−	+	+	(4)	(11)	−
	2	−	+	+	+	(9)	−
	3	−	+	+	(4)	(1)	(1)
PowerPlex Y	1	−	+	+	(4)	(11)	−
	2	−	+	+	+	(6)	−
	3	−	+	+	(10)	(3)	−
Y-Plex 12	1	−	+	+	−	−	−
	2	−	+	−	(4)	−	−
	3	−	NA	NA	NA	NA	NA
Multiplex I	1	−	+	+	+	−	−
	2	−	+	+	+	−	−
	3	−	NA	NA	NA	NA	NA
Multiplex B	1	−	+	+	+	−	−
	2	−	+	+	+	−	−
	3	−	NA	NA	NA	NA	NA

+, full profile; −, no profile; (#), partial profile with number of typeable loci indicated; NA, not applicable (i.e., not tested).

DNA Isolation with a NonDifferential Extraction Method

The use of 300 ng of nondifferentially extracted DNA produced complete Y-STR profiles 3 days postcoitus with all the Y-STR systems tested (data not shown). With few exceptions, the 4- to 6-day samples subjected to a nondifferential extraction procedure detected fewer loci (both with commercial kits and in-house systems) than a differential lysis procedure carried out on the same samples (Table 2 and data not shown).

Effect of Post-PCR Clean Up

Prior to capillary electrophoresis the amplified sample is electrokinetically injected into the capillary, effectively drawing negatively charged molecules such as DNA into the capillary. The short injection time (typically 5 sec) permits a limited amount of sample to be taken into the capillary. In this process the uptake of smaller components is favored; STR amplimers compete with primers, unincorporated dNTPs, salts, and other negatively charged PCR reaction components. In theory, the removal of unincorporated amplification components should favor amplicon injection and lead to an increase in fluorescent signal intensity. This laboratory has previously demonstrated the enhanced sensitivity of STR amplicon detection using a post-PCR clean up process based upon the differential binding of amplimer DNA to silica columns (29).

Yfiler™ and PowerPlex® Y amplification products from 3–7 days postcoital samples were subjected to post-PCR clean up using MinElute™ silica columns prior to electrophoretic separation and analysis. The results are summarized in Table 2. In most instances post-PCR purification resulted in increases in allelic signal intensities and, for those samples exhibiting a partial profile, an increase in the number of typeable loci. The improvement in typing efficiency was observed in samples subjected to a differential lysis or a nondifferential extraction methodology and, in some cases, the enhancement was substantial. For example, an 11 locus partial Yfiler™ profile from a 6-day postcoital differentially extracted sample gave a full 17 locus profile upon MinElute™ purification (Table 2, couple #1).

Remarkably a partial 8 locus Y-STR profile was obtained with Yfiler™ from a 7-day postcoital sample after post PCR purification (Fig. 3*b*), when double the usual quantity of DNA was amplified (10 μL vs. 5 μL). Without post-PCR purification, no profile was obtained (Fig. 3*a*).

Discussion

The impetus for the studies described in this report was the knowledge that male cells are present in the cervix at least a week after intercourse and yet standard DNA typing systems are unable to discern the male donor profile after 3 days or less. Here we report the ability to obtain a Y-STR profile from the male donor in extended interval (≥3 days) postcoital samples. Our previous work demonstrated that the proper collection of cervicovaginal samples (as opposed to the lower or mid-tract vaginal canal samples often taken) is critical for optimal recovery of sperm for analysis (1). In this study, using cervicovaginal swabs collected from volunteers, we have demonstrated the relative facility of obtaining full Y-STR profiles from cervicovaginal samples recovered 3–4 days after intercourse using both commercial and in-house systems. Profiles were also obtainable 5–6 days postcoitus although by this stage partial profiles rather than full profiles were a more likely outcome. Almost without exception, more complete profiles were obtained when DNA from the sperm fraction of a differential lysis method was amplified as opposed to the amplification of relatively large

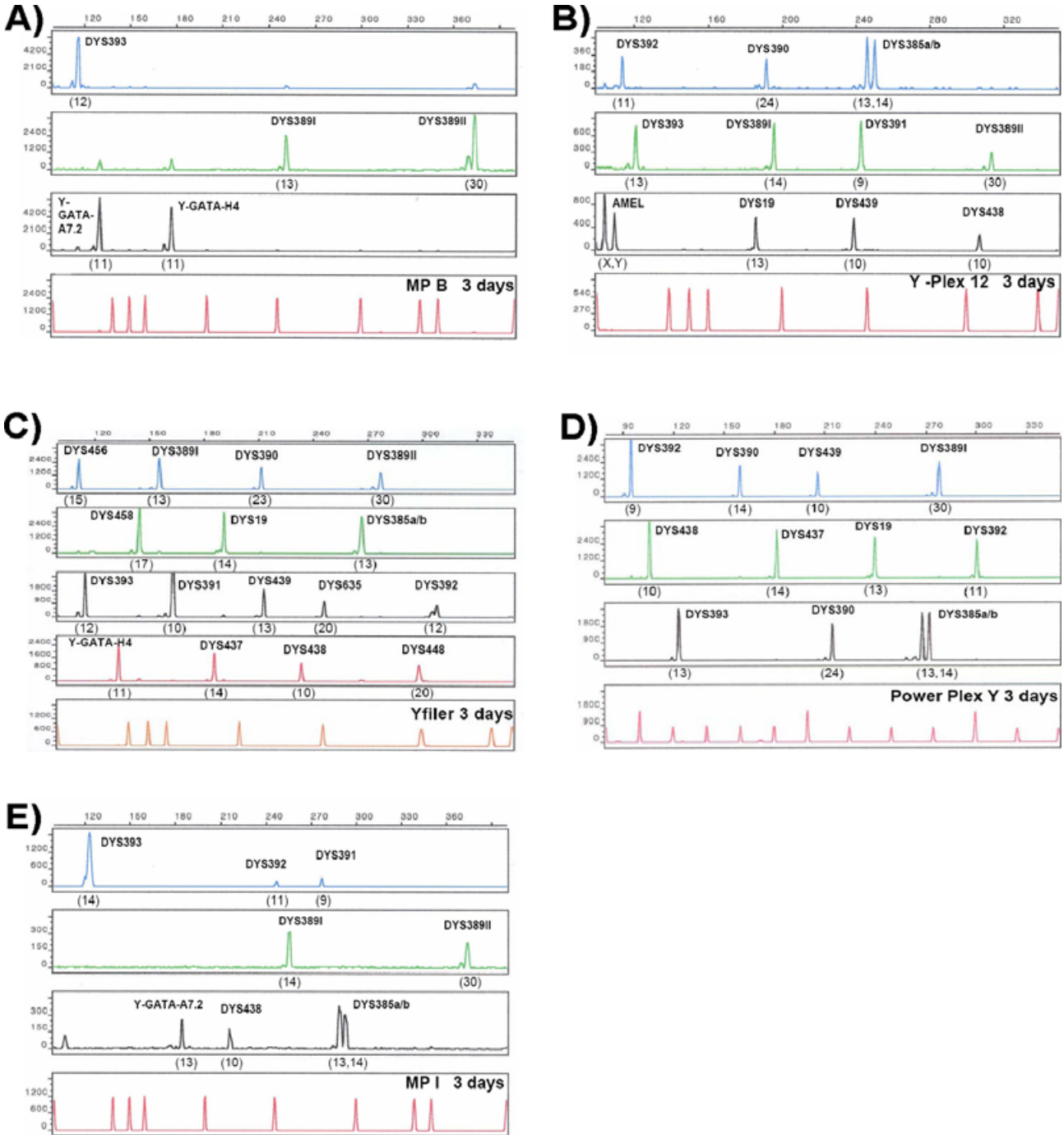

FIG. 1—*Y-STR profiles from 3-day postcoital samples. Samples were subjected to differential lysis and 1 ng of the sperm fraction amplified. (A) MPB, (B) Y-PLEX™ 12, (C) AmpFℓSTR® Yfiler™, (D) PowerPlex® Y, (E) MPI. Allele designations for each locus are provided.*

quantities (i.e., *c.* 300 ng) of admixed male/female DNA. The latter diminution of efficacy may be due to the loss of critical PCR reaction components by titration in the presence of contaminating female DNA. Additionally, the DNA profiles from the sperm fraction of a differential lysis were of superior quality to that obtained when a nondifferential method was employed in that the allelic signal intensities were generally higher and more balanced, and exhibited less baseline noise.

Importantly, the incorporation of a simple post-PCR purification process significantly increased the ability to obtain Y-STR profiles, particularly from 5- to 6-day postcoital samples. Post-PCR purification increases the sensitivity of allele detection resulting not only in increased peak heights with partial profiles but also the appearance of loci that otherwise were not apparent in the nonpurified sample. For example, using differential lysis and MinElute purification, profiles with 10–17 locus were obtained from 6-day postcoital samples that, prior to purification gave 6–11 locus profiles. Incredibly, an 8 locus Y-STR profile was obtained from a 7-day postcoital sample, which is approaching the time interval limit for sperm detection in the cervix reported in the medical literature.

The two commonly used commercial Y-STR systems, namely AmpFℓSTR® Yfiler™ and PowerPlex® Y, performed extremely

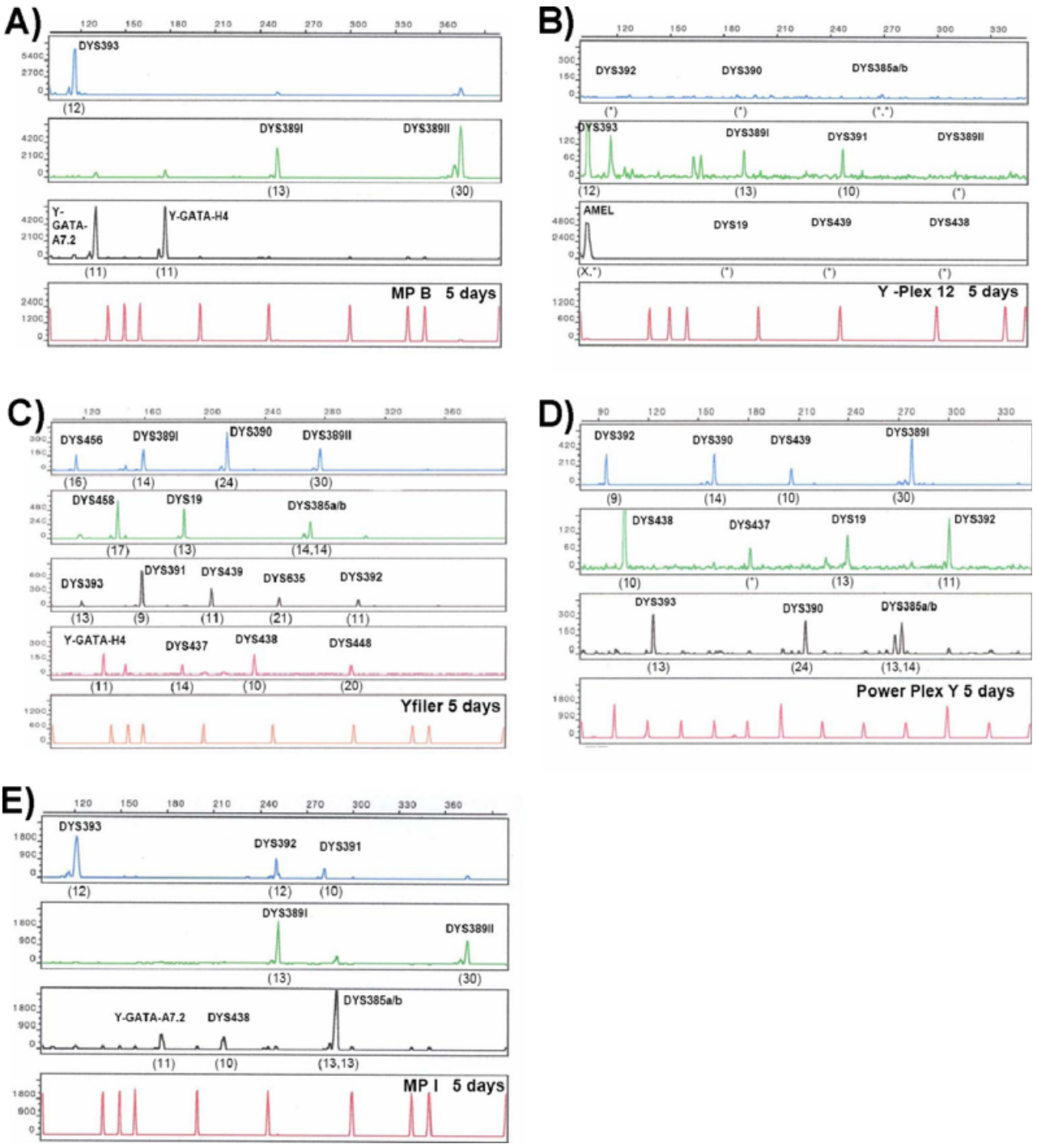

FIG. 2—*Y-STR profiles from 5-day postcoital samples. Samples were subjected to differential lysis and 1 ng of the sperm fraction amplified. (A) MPB (B) Y-PLEX™ 12, (C) Yfiler™, (D) PowerPlex® Y, (E) MP1. Allele designations for each locus are provided. Allele drop out is represented by (*).*

well particularly when considering that the manufacturer's recommended PCR amplification conditions were used throughout. Indeed they performed better than our in-house "benchmark" systems with 6-day postcoital samples since no profiles at all were obtained with MPI and MPB. The third commercial system evaluated, Y-PLEX™ 12, was found not to be particularly suited for >4-day postcoital samples. We speculate that this may be due to the incorporation of the AMEL marker, which is likely to result in titration of critical PCR reaction components in the presence of contaminating female DNA. In any case the Y-PLEX™ 12 kit is no longer generally available to the public.

We intend to pursue additional studies that may further enhance our ability to obtain good quality and probative DNA profiles from extended interval postcoital samples. Such studies will include the use of increased cycle number (31,32), Y-STRs with reduced amplimer sizes ("mini-Y-STRs") (33,34), and improved cervicovaginal sample collection devices. Revisiting the possibility of autosomal STR profiling of extended interval postcoital samples using similar strategies may also be worthwhile.

Finally, the results of the studies reported here could have dramatic effects on how rape cases are investigated and prosecuted. Currently, vaginal samples are often only taken from the victim up

TABLE 2—*Effect of post-PCR purification on Y-STR typing of postcoital samples.*

		Postcoital Interval (Days)							
		4		5		6		7	
Y-STR System	DNA Extraction	No MinElute	MinElute	No MinElute	MinElute	No MinElute	Min Elute	No MinElute	Min Elute
Y-filer	ND[1]	(13)	(12)	–	–	–	(4)	–	(1)
	D[1]	+	+	(4)	(6)	(11)	+	–	(1)
	ND[2]	+	+	(14)	(6)	(1)	(11)	–	–
	D[2]	+	+	+	+	(9)	(10)	–	(1)
PowerPlex Y	ND[1]	(5)	(7)	(1)	–	(3)	(2)	(1)	–
	D[1]	+	+	(4)	(6)	(10)	+	–	(1)
	ND[2]	+	+	(8)	+	(1)	(2)	(2)	–
	D[2]	+	+	+	+	(6)	(10)	–	(3)

+, full profile; –, no profile; (#), partial profile with number of typeable loci indicated; ND#, nondifferential extraction with samples from couple number #; D#, differential lysis with samples from couple number #

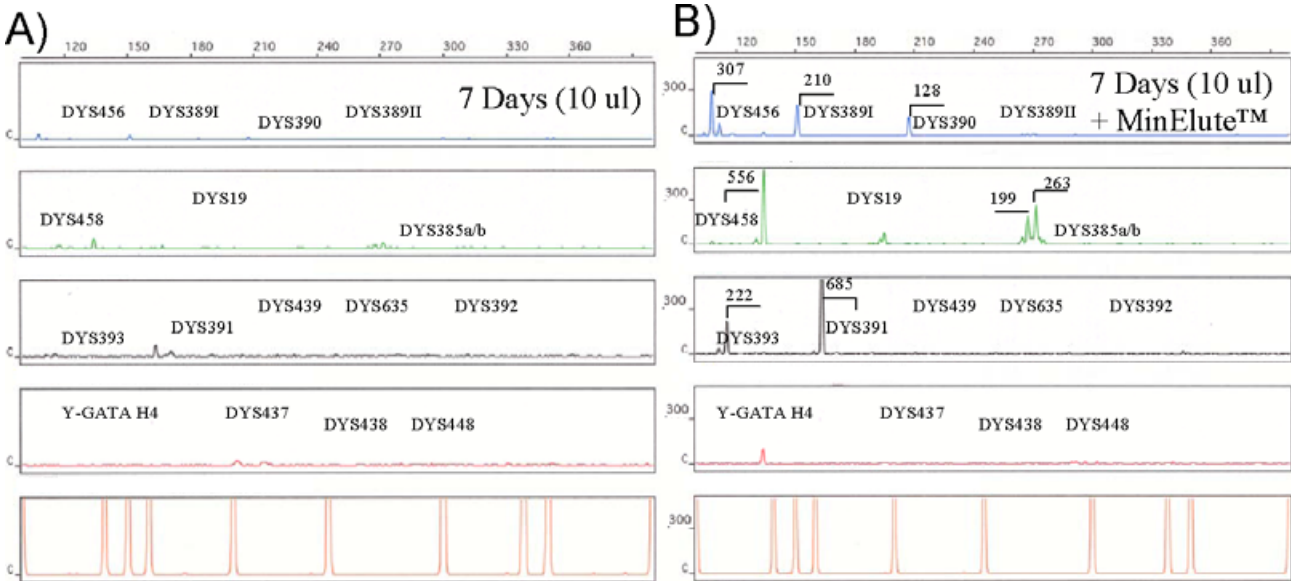

FIG. 3—*Y-STR Profile obtained from a 7-day postcoital sample. The sample was subjected to differential lysis and 10 μL of the sperm fraction amplified using Yfiler™. (a) Without post-PCR purification, (b) with MinElute™ post PCR purification.*

to 72 h after the sexual assault. However, our results indicate that potential evidence may be lost if this practice continues. Thus changes in the way rape kit evidence is recovered may be necessary in some jurisdictions, including specific instructions on how to conduct cervicovaginal sampling and how long after the assault to take the samples.

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References

1. 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;3:58–72.
2. Davies A, Wilson E. The persistence of seminal constituents in the human vagina. Forensic Sci 1974;3(1):45–55.
3. Eungprabhanth V. Finding of the spermatozoa in the vagina related to elapsed time of coitus. Z Rechtsmed 1974;74(4):301–4.
4. Morrison AI. Persistence of spermatozoa in the vagina and cervix. Br J Vener Dis 1972;48(2):141–3.
5. Rupp JC. Sperm survival and prostatic acid phosphatase activity in victims of sexual assault. J Forensic Sci 1969;14(2):177–83.
6. Willott GM, Allard JE. Spermatozoa—their persistence after sexual intercourse. Forensic Sci Int 1982;19(2):135–54.
7. Perloff WH, Steinberger E. In vivo survival of spermatazoa in cervical mucus. Am J Obstet Gynecol 1964;88:439–42.
8. Sharpe N. The significance of spermatazoa in victims of sexual offences. Can Med Assoc J 1963;89:513–4.
9. Silverman EM, Silverman AG. Persistence of spermatozoa in the lower genital tracts of women. JAMA 1978;240(17):1875–7.
10. Silverman EM, Silverman AG. Presence of spermatozoa in cervicovaginal smears from young and old women. Exp Aging Res 1979;5(2):155–9.
11. Collins PJ, Hennessy LK, Leibelt CS, Roby RK, Reeder DJ, Foxall PA. Developmental validation of a single-tube amplification of the 13 CODIS STR loci, D2S1338, D19S433, and amelogenin: the AmpFlSTR Identifiler PCR Amplification Kit. J Forensic Sci 2004;49(6):1265–77.
12. Finis CJ. Megaplex STR analysis from a single amplification: validation of the PowerPlex 16 System. Profiles in DNA 2001;4(2):5–8.

13. Frank WE, Llewellyn BE, Fish PA, Riech AK, Marcacci TL, Gandor DW, et al. Validation of the AmpFlSTR Profiler Plus PCR amplification kit for use in forensic casework. J Forensic Sci 2001;46(3):642–6.
14. Holt CL, Buoncristiani M, Wallin JM, Nguyen T, Lazaruk KD, Walsh PS. TWGDAM validation of AmpFlSTR PCR amplification kits for forensic DNA casework. J Forensic Sci 2002;47(1):66–96.
15. LaFountain MJ, Schwartz MB, Svete PA, Walkinshaw MA, Buel E. TWGDAM validation of the AmpFlSTR Profiler Plus and AmpFlSTR COfiler STR multiplex systems using capillary electrophoresis. J Forensic Sci 2001;46(5):1191–8.
16. Daniels DL, Hall AM, Ballantyne J. SWGDAM developmental validation of a 19-locus Y-STR system for forensic casework. J Forensic Sci 2004;49(4):668–83.
17. Hall A, Ballantyne J. The development of an 18-locus Y-STR system for forensic casework. Anal Bioanal Chem 2003;376(8):1234–46.
18. Hanson EK, Ballantyne J. A highly discriminating 21 locus Y-STR ''megaplex'' system designed to augment the minimal haplotype loci for forensic casework. J Forensic Sci 2004;49(1):40–51.
19. Hanson EK, Berdos PN, Ballantyne J. Testing and evaluation of 43 ''noncore'' Y chromosome markers for forensic casework applications. J Forensic Sci 2006;51(6):1298–314.
20. Parson W, Niederstatter H, Brandstatter A, Berger B. Improved specificity of Y-STR typing in DNA mixture samples. Int J Legal Med 2003;117(2):109–14.
21. 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(3):288–91.
22. Yoshida Y, Fujita Y, Kubo S. Forensic casework of personal identification using a mixture of body fluids from more than one person by Y-STRs analysis. J Med Invest 2004;4:238–42.
23. Mulero JJ, Chang CW, Calandro LM, Green RL, Li Y, Johnson CL, et al. Development and validation of the AmpFlSTR Yfiler PCR amplification kit: a male specific, single amplification 17 Y-STR multiplex system. J Forensic Sci 2006;51(1):64–75.
24. Krenke BE, Viculis L, Richard ML, Prinz M, Milne SC, Ladd C, et al. Validation of a male-specific, 12-locus fluorescent short tandem repeat (STR) multiplex. Forensic Sci Int 2005;148(1):1–14.
25. Shewale JG, Nasir H, Schneida E, Gross AM, Budowle B, Sinha SK. Y-chromosome STR system, Y-PLEX 12, for forensic casework: development and validation. J Forensic Sci 2004;49(6):1278–90.
26. Comey CK, Presely KW, Smerick JB, Sobieralski CA, Stanley DM, Baechtel FS. Extraction strategies for amplified fragment length polymorphism analysis. J Forensic Sci 1994;39:1254–69.
27. Green RL, Roinestad IC, Boland C, Hennessy LK. Developmental validation of the quantifiler real-time PCR kits for the quantification of human nuclear DNA samples. J Forensic Sci 2005;50(4):809–25.
28. Mayntz-Press KA, Ballantyne J. Performance characteristics of commercial Y-STR multiplex systems. J Forensic Sci 2007;52(5):1025–34.
29. Smith PJ, Ballantyne J. Simplified low-copy-number DNA analysis by post-PCR purification. J Forensic Sci 2007;52(4):820–9.
30. Hall A, Ballantyne J. Strategies for the design and assessment of Y-STR multiplexes for forensic use. Forensic Sci Rev 2003;15:137.
31. Gill P. Application of low copy number DNA profiling. Croat Med J 2001;42(3):229–32.
32. Whitaker JP, Cotton EA, Gill P. A comparison of the characteristics of profiles produced with the AMPFlSTR SGM Plus multiplex system for both standard and low copy number (LCN) STR DNA analysis. Forensic Sci Int 2001;3:215–23.
33. Park MJ, Lee HY, Chung U, Kang SC, Shin KJ. Y-STR analysis of degraded DNA using reduced-size amplicons. Int J Legal Med 2007;121(2):152–7.
34. Tsukada K, Takayanagi K, Asamura H, Ota M, Kobayashi K, Fukushima H. Simultaneous PCR of eight loci for very short Y-STR fragment size. Prog Forensic Genet 2004;10:322–4.

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