

Analysis of DNA Evidence Recovered from Epithelial Cells in Penile Swabs

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In the rape case presented here, no semen, hair, or other biological evidence were left by the perpetrator at the crime scene or on the victim. The alleged assailant was arrested soon after the crime. A classical stain recovery technique using cotton swab moistened with sterile water was taken for recovering potential female epithelial cells and leukocytes deposited on the alleged assailant's penis during sexual assault. The organic method used for DNA extraction was quantified according to the slot-blot procedure and amplified at 9 and 15 polymorphic loci. Penile swab revealed a DNA profile of mixed origin. In addition to the suspect's DNA profile, DNA contribution from the victim was identified as a minor component in the mixture. Frequency of the profile resulted in a value of 5×10^{-14} for the multiplex systems AmpFISTR Plus and 2.5×10^{-18} for the multiplex system PowerPlex 16, taking into account only non-overlapping alleles between the suspect and the victim from the minor component in the DNA mixture. Moreover, three additional alleles were observed at D21S11 locus by use of PowerPlex® and STR SGM® plus primers, which could not belong to the suspect. The victim's DNA profile showed the same three-banded genotype at this locus. The same pattern was detected when the victim's saliva or blood were used as reference samples. Our laboratory finding was consistent with the police report that the victim was a person with Down syndrome, a human genetic disease mainly resulting from trisomy (triplication) of the 21 chromosome.

Key words: alleles; DNA fingerprinting; Down syndrome; forensic medicine; polymerase chain reaction; rape; sex offences; specimen handling; tandem repeat sequences

Traditionally, the finding of semen in forensically significant specimens, such as cervicovaginal samples or semen stains on the victim's clothes, has been considered the positive evidence needed to prove sexual contact. In the absence of spermatozoa, other tests, such as acid phosphatase or prostate-specific antigen assay test, can be used to detect the presence of semen (1,2). Unfortunately, in many sexual assault or rape cases no spermatozoa or semen stains can be found. Application of Y-chromosome short tandem repeat testing (Y-STR) in forensic casework can be very useful when a mixture of female and male DNA is present in the sample, especially when DNA of the male contributor is present in a very small amount (e.g., in vaginal swabs) (3). However, the amplification of Y-STRs is known to produce non-Y-chromosomal amplification products, which most likely derive from a high level background of female DNA (4). Instead of collecting only the stains from the body of the victims, many investigators today consider that the vaginal cells deposited on the suspect's penis during sexual assault are an important alternative focus in cases of sexual abuse and assault. It is known that cells shed from a female during sexual intercourse can be retrieved from the penis within a 48-hour post-

coital period (5). The sensitivity of DNA typing based on polymerase chain reaction (PCR), which allows minute traces of DNA evidence to be analyzed even when partially degraded, provides significant advantage (6,7).

Autosomal STR multiplex and megaplex systems have become very useful especially in the analysis of mixed stains consisting of a combination of cells (vaginal and buccal epithelial cells) and physical fluids (vaginal secretion and saliva) that cannot be separated by use of preferential lysis method. DNA analysis of this kind of samples has to be performed with a kit containing as many polymorphic loci as possible to provide powerful discrimination. In these cases, it is very important to take a penile swab from the suspect, because this can be the only evidence proving the contact between the victim and the suspect.

Material and Methods

Description of the Assault

An 18-year-old mentally retarded girl was attacked in a stable by an older man, a farm worker hired by victim's father to help the family at the farm. Immediately after the crime, the victim went to find her mother and complained about what the man did to her. At the crime scene, the police did not find any evi-

dence. They only collected the victim’s clothing worn during the assault. The victim was sent to the hospital where sexual assault evidence (cervical and vaginal samples from victim) was taken during a physical examination. The suspect was arrested the same day. A young police officer decided to swab the suspect’s penis with moistened cotton swab for collecting potential biological evidence, as recommended in our police guidelines for collection and preservation of DNA evidence.

Preparation and Quantification of DNA

Buccal epithelial cells from the suspect and the victim were collected by use of cotton swabs from C.D.S. Swab Safe Box (Swissforensix AG, Bern, Switzerland). Blood sample was also taken from the victim as the second reference sample. Nuclear DNA from these samples was extracted with 5% Chelex suspension according to the standard method (8). Penile swab was submitted to proteolytic digestion followed by organic extraction and Centricon®-100 filtration (9). DNA was determined by slot-blot hybridization with primate-specific D17Z1 alpha-satellite probe by use of QuantiBlot® assay (Applied Biosystem, Foster City, CA, USA) (10).

Amplification

Autosomal STRs and the gender specific marker, Amelogenin, were typed by use of the AmpFISTR® SGM Plus® Amplifi-

cation kit (Applied Biosystem) and the PowerPlex® 16 System (Promega, Madison, WI, USA), according to the manufacturers’ protocols (11,12). Amplifications were performed on the Perkin-Elmer 9600 (Applied Biosystem).

Detection and Data Analysis

Typing of PCR products was performed on the ABI PRISM® 377 sequencer (Applied Biosystem) with 5% Long Ranger® gels (BioWhittaker Molecular Applications, Rockland, ME, USA). Data from samples amplified with AmpFISTR® SGM Plus PCR Amplification kit were collected by ABI PRISM Collection software with virtual filter set F. Genotyper analysis was defined by KAZAM macro provided with the Genotyper 2.5 software (Applied Biosystem), with a minimum allele detection threshold set at 75 relative fluorescence units (rfu) as recommended by the AmpFISTR User’s Manual (11). Data from samples amplified with PowerPlex PCR Amplification kit were collected by use of ABI PRISM Collection software with virtual filter set A. Results were analyzed with GeneScan analysis software and PowerTyper™ Macro software program (Promega), with a minimum threshold for allele detection set between 150-50 rfu (12).

Interpretation of Mixture

The interpretation of the results was done according to the principles outlined by Gill et al (13) and Clayton et al (14).

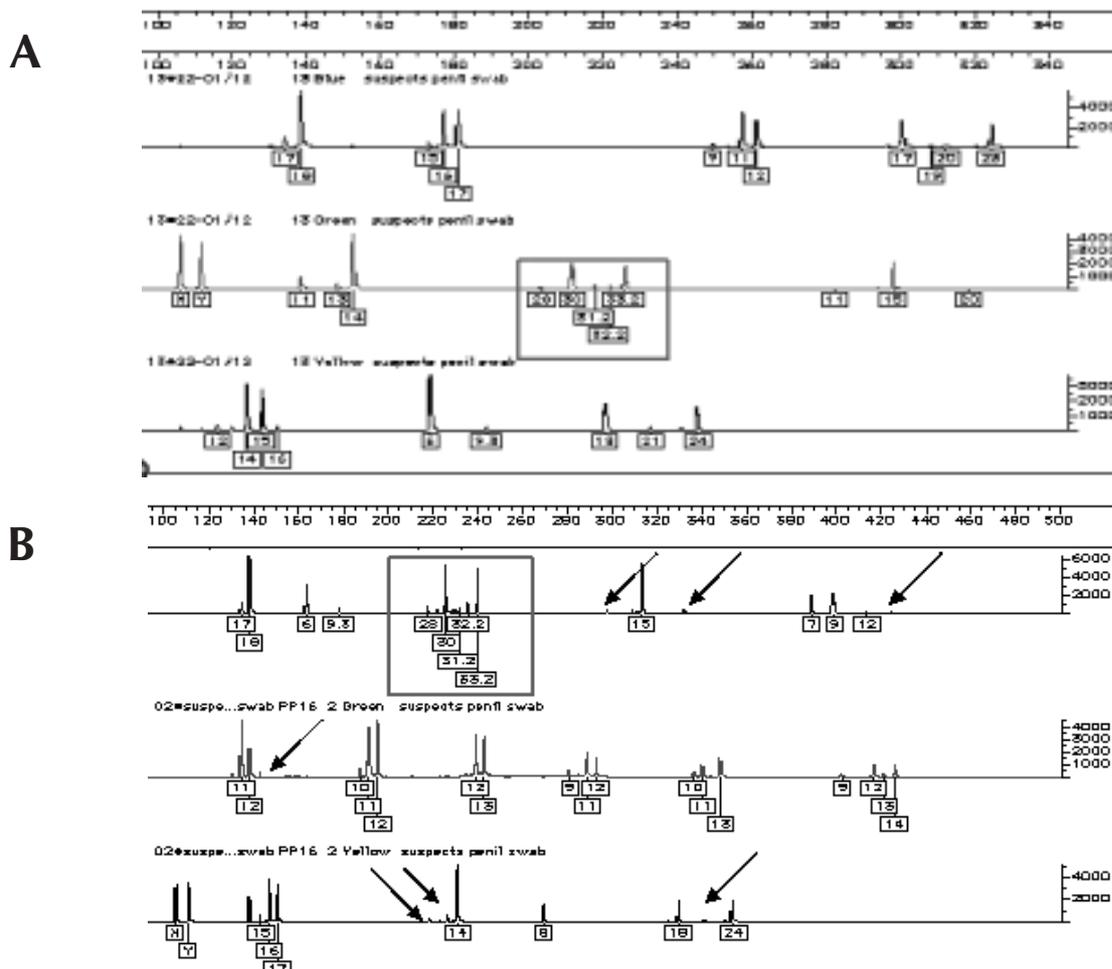


Figure 1. Electropherograms of AmpFISTR® SGM Plus® (A) and PowerPlex® 16 system (B) amplified DNA from the penile swabs showing a mixture with a dominant male component. Several minor peaks are visible, but some of them (D18S51, Penta E, D5S818, D8S51, and FGA) are not labeled at the threshold value of 150 rfu on PowerPlex® 16 (arrows), as routinely used in our laboratory, whereas for the AmpFISTR® SGM Plus the default minimum threshold is set at 75 rfu. When we reanalyzed the amplified samples from PowerPlex® 16 at a minimum threshold of 50 rfu, all the alleles marked with the arrows became labeled (data not shown). The profile from the minor component could not be distinguished from that of the victim’s. The gray box indicates the same three-band profile observed at locus D21S11 in the sample from the victim (Figs. 2 and 3) and in the minor component of the suspect’s penile swabs.

Results

No seminal fluid and/or sperm cells could be detected in any of the items of evidence taken from the victim (clothes and cervicovaginal samples). The amount of DNA recovered from each of the two swabs taken from the suspect’s penis was below the lowest DNA reference standard (0.15 ng). Consequently, 200 µL of both extracts were pooled and concentrated on Microcon®-100 filters up to 50 µL. Then, 10 µL of DNA extract from pooled sample was successfully amplified with the AmpFISTR® SGM Plus. Multilocus profile obtained from the penile swabs indicated mixed STR profile by the presence of more than two bands at some loci (Fig. 1A). Since the number of extra allelic peaks did not exceed four peaks, with the exception of locus D21S11, we assumed that the mixture contained DNA from two persons. To obtain more information, seven additional loci were analyzed with PowerPlex® 16 using the same amount of DNA sample (Fig. 1B). The number of the bands at any locus did not exceed four. It was possible to separate the major and the minor component by visual examination. The alleles in the minor component were specific for the victim (Fig. 2A and

3A), whereas the major component matched the suspect (Fig. 2B and 3B). However, in the case of amplification with PowerPlex® 16, some alleles were not labeled, but the signals were detectable (Fig. 1B).

In our laboratory, we routinely analyze DNA amplified with AmpFISTR® Plus at 75 rfu cut-off; the minimum threshold for DNA amplified by Powerplex® 16 is set at 150 rfu. The reason for such a practice is that we have much more experience with DNA samples amplified with AmpFISTR® kit than with PowerPlex® kit. However, we reanalyzed the same DNA sample from the penile swab at the threshold set at 50 rfu, and alleles specific for the victim were labeled at all loci, except for the loci D7S820 and TPOX, where the genotype of the suspect and the victim were the same (Table 1). At this threshold, determined alleles could still be clearly distinguished from the background (data not shown). Compared with the loci analyzed with the AmpSTR® Plus, the PowerPlex® 16 included two highly polymorphic pentanucleotide loci (Penta E and Penta D) with reduced stutter peaks, which could additionally help in the interpretation of the DNA mixture. Assuming that the major component was from the suspect, the estimated frequency of the occurrence of the minor component deduced from

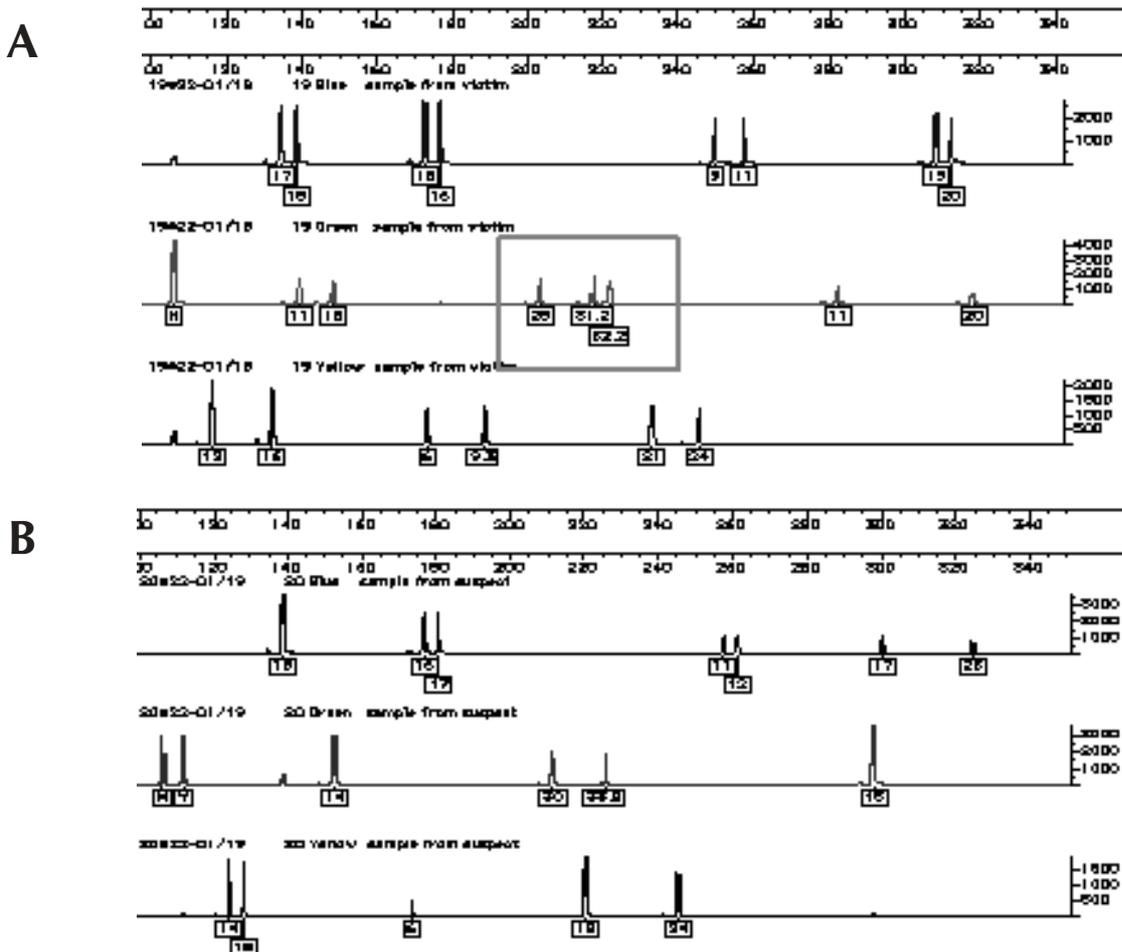


Figure 2. Electropherogram of the reference samples from the victim (A) and the suspect (B) amplified by AmpFISTR® SGM Plus®. The gray box indicates the same three-band profile observed at locus D21S11 in the sample from the victim and in the minor component of the suspect’s penile swabs.

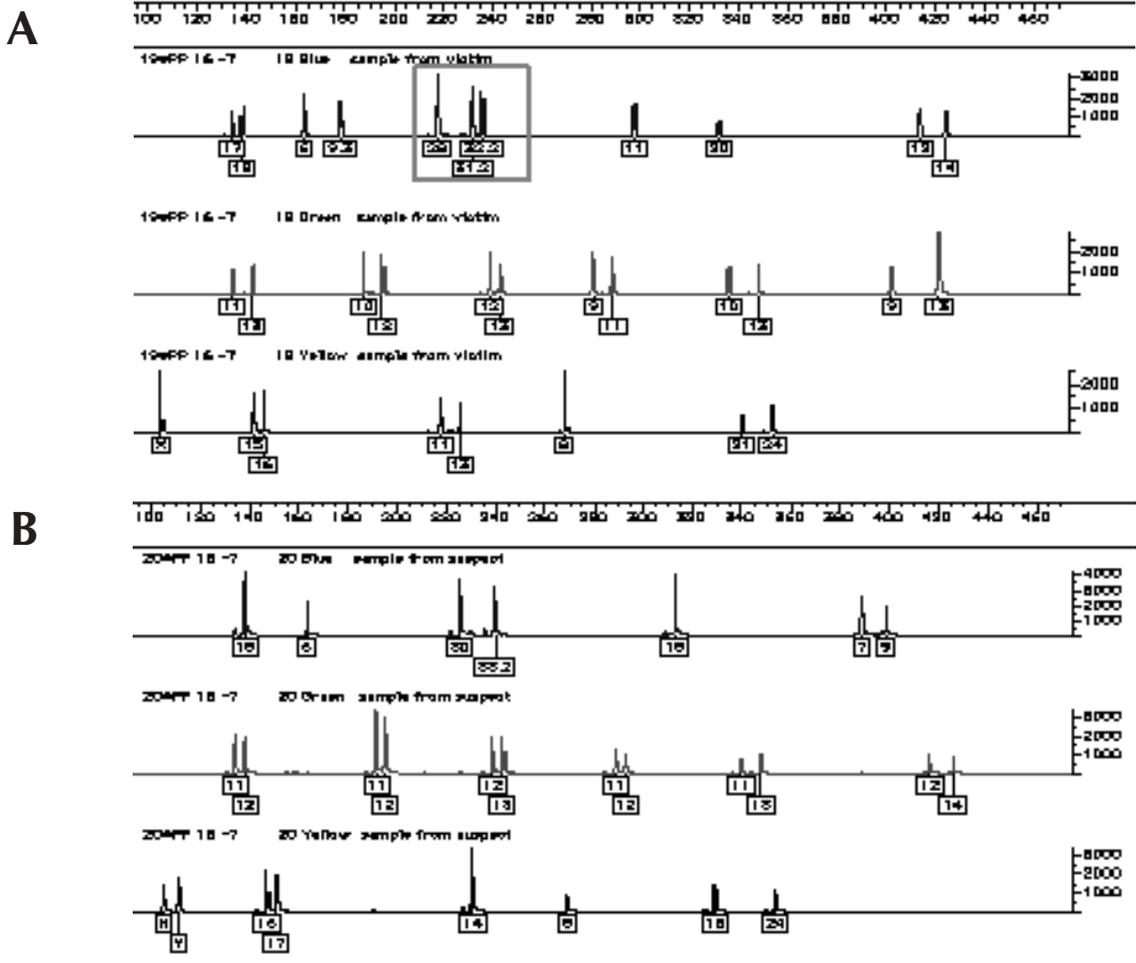


Figure 3. Electropherogram of the reference samples from the victim (A) and the suspect (B) amplified by PowerPlex® 16 system. The gray box indicates the same three-band profile observed at locus D21S11 in the sample from the victim and in the minor component of the suspect’s penile swabs.

the non-overlapping alleles between the suspect and the victim (Table 1) for 9 and 15 STR loci in the Slovenian population is approximately 5×10^{-14} and 2.5×10^{-18} , respectively (15,16). In addition, the profile from the minor component at D21S11 locus (Fig. 1) showed the same three-band pattern as the victim’s profile (Fig. 2A and 3A), which further increased the evidential value of DNA analysis. On the basis of the rarity of the minor component profile, together with the presence of the same genetic anomaly in the minor component in the forensic sample as well as in the reference samples from the victim, it was determined that the origin of DNA from the minor component in the suspect’s penile swab and from the victim was the same.

Discussion

The suspect’s penile swabs can be used as potential source of DNA evidence. PCR-based analysis of DNA from different epithelial cells deposited on the human skin and recovered by swab technique has already been described (17). This type of evidence re-

mains stable on intact skin for at least 48 hours after deposition (5).

In our case, the quantity of DNA recovered from the penile swab taken from the suspect by the single swab technique was relatively low. It is unclear whether this was due to the low amount of trace material or because the double swab technique was not used (18). However, investigators should think of biological evidence that can be found on the suspect in sex offences and sexual harassment cases and routinely swab the penis of an alleged assailant for recovering potential evidence indicative of sexual assault, since the perpetrator is often cautious not to leave the sperm on the victim’s body.

In our case, the nuclear DNA we recovered from the suspect’s penis was of sufficient quality and quantity to perform multiplex STR analysis. No other biological evidence was found. In the postcoital samples no spermatozoa were detected. The penile swab was the only physical and biological evidence that could prove the contact between the suspect and the victim. The victim’s DNA profile was consistent with that of the female epithelial cells deposited on the suspect’s

Table 1. STR typing results of the suspect's penile swabs and the reference samples from the victim and the suspect*

Locus	Victim	Penile swab	Suspect
STR SGM Plus kit:			
D3S1358	17, 18	17, 18	18, 18
VWA	15, 16	15, 16, 17	16, 17
D16S539	9, 11	9, 11, 12	11, 12
D2S1338	19, 20	17, 19, 20, 23	17, 23
D8S1179	11, 13	11, 13, 14	14, 14
D21S11	28, 31.2, 32.2	28, 30, 31.2, 32.2, 33.2	30, 33.2
D18S51	11, 20	11, 15, 20	15, 15
D19S433	12, 16	12, 14, 15, 16	14, 15
THO1	6, 9.3	6, 9.3	6, 6
FGA	21, 24	18, 21, 24	18, 24
Amelogenin	XX	XY	XY
Powerplex 16 kit:			
D3S1358	17, 18	17, 18	18, 18
THO1	6, 9.3	6, 9.3	6, 6
D21S11	28, 31.2, 32.2	28, 30, 31.2, 32.2, 33.2	30, 33.2
D18S51	11, 20	11, 15, 20	15, 15
Penta E	12, 14	7, 9, 12, 14	7, 9
D5S818	11, 13	11, 12, 13	11, 12
D13S317	10, 12	10, 11, 12	11, 12
D7S820	12, 13	12, 13	12, 13
D16S539	9, 11	9, 11, 12	11, 12
CSF1PO	10, 13	10, 11, 13	11, 13
Penta D	9, 13	9, 12, 13, 14	12, 14
VWA	15, 16	15, 16, 17	16, 17
D8S1179	11, 13	11, 13, 14	14, 14
TPOX	8, 8	8	8, 8
FGA	21, 24	18, 21, 24	18, 24
Amelogenin	XX	XY	XY

*Alleles given in bold letters represent peaks that could be assigned to the victim.

penis. In addition, the unusual three-band pattern unequivocally detected at the STR locus on 21 chromosome in the minor component of DNA mixture from the suspect's penile swab was the same as the pattern we observed in both victim's reference samples. Our finding was in concordance with the police report stating that the victims was a person with Down syndrome, a human disease resulting from trisomy (triplication) of the 21 chromosome (19).

The present example also shows a great usefulness of multiplex PCR technique for the identification of mixed biological evidence, especially when the amount of DNA is low. The data indicated that the AmpFISTR® SGM Plus® Kit and PowerPlex® 16 System are extremely sensitive multiplex STR amplification systems.

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Received: January 29, 2003

Accepted: April 22, 2003

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