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CASE REPORT

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DNA analysis of fingernail debris using different multiplex systems: a case report

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Abstract A 55-year-old male nurse was accused of having introduced his fingers by force into the anus of a 20-year-old female patient. Debris from the fingernails of the suspect recovered 2 days after the incident was analysed with the VNTR locus D1S80, the triplex PCR system AmpF*l*STR Blue kit, the AmpF*l*STR Profiler kit and the pentaplex system genRES MPX. The D1S80 singleplex reaction revealed indications of DNA from the victim in the fingernail debris of the left hand. Using the AmpF*l*STR Blue kit and AmpF*l*STR Profiler, DNA alleles of the victim were found at four additional loci, while allelic drop-out was observed at five other loci. Only the pentaplex kit genRES MPX revealed alleles at all loci which could be assigned to the victim. Calculation of likelihood ratios resulted in a value of 1.4×10^5 using the combination of the multiplex systems AmpFlSTR Blue kit and AmpF*l*STR Profiler and 2.8×10^8 for the genRES MPX kit. This case demonstrates the high sensitivity of the new genRES MPX kit and that DNA profiling of fingernail debris is possible despite a time lapse of 2 days between the incident and recovery of the evidential material.

Keywords Multiplex · Fingernails · STR · DNA typing

Introduction

Violent crimes and sexual assault cases are often associated with multiple actions of aggression and defence which may lead to transfer of DNA-containing material. This material could possibly originate from contact with blood and other body fluids such as saliva, semen or mucus. In addition, scratching may leave traces of the stratum corneum and upper epidermal layers under the fingernails of the victim or the suspect (Wiegand et al. 1993). Therefore, in relevant cases the analysis is focused on fingernail clippings or on debris scraped from underneath nails (Keating and Allard 1994). In most cases the majority of the material is DNA originating from the fingernails themselves, leading to the problem of identification of a secondary source of DNA which is quantitatively underrepresented (Oz and Zamir 2000). Nevertheless, PCR amplification provides a powerful tool even in cases of mixed traces containing highly unbalanced DNA amounts from different donors (Wallin et al. 1998; Lederer et al. 2000). In addition, PCR multiplex systems have been developed which contain highly discriminating STR loci with a high specificity and sensitivity. Thus, these systems produce results with a high evidential value even in cases of limited evidential material (Kimpton et al. 1993; Urquhart et al. 1995; Rolf et al. 1997; Lee et al. 1998). In this report the capability of three multiplex STR systems, AmpF*l*STR Profiler, AmpF*l*STR Blue kit (Perkin Elmer Applied Biosystems, Weiterstadt, Germany) and genRES MPX (Serac, Bad Homburg, Germany) and one VNTR system (D1S80) is demonstrated, to analyse fingernail debris recovered from the suspects fingernails 2 days after a sexual assault. According to the statement of the suspect, who denied the assault, he had washed his hands several times during this time period.

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Case report

A 20-year-old woman was admitted to hospital under the suspicion of an alcohol intoxication. The woman was unable to communicate and showed no reaction to pain stimulus. After the first examination by a physician, vital functions were monitored and a venous access was performed. Using administrative tasks as an excuse for being alone with the patient, a 55-year-old male nurse sent his female colleagues out of the emergency room. According to the later testimony of the victim, the man manipulated her genital region and introduced one or more fingers into her anus. Due to this painful action, the woman awoke from her somnolent state and tried to push the nurse away. Alarmed by shouts from the room, two nurses came in and saw the male nurse standing near by the woman completely taken aback. The victim immediately accused the man of having manipulated her sexually.

After releasing herself from the clinic in the early next morning, the woman reported the nurse to the police. A gynaecological examination was performed and a blood sample was taken. After a time lapse of 2 days the fingernail debris of the alleged offender was recovered for DNA analysis. The nurse stated that he had repeatedly washed his hands during this time period and had never been in contact with blood or other body fluids of the young woman.

Material and methods

Fingernail debris of the alleged offender was scraped out with a small plastic spatula and collected separately for each hand. Bloodstains and fingernail debris were extracted with Chelex 100 (Bio-Rad) in a total volume of 200 µl (Walsh et al. 1991) without subsequent quantitation of the DNA amount. For fingernail debris, 2 µl of proteinase K solution (20 mg/ml) was added and incubated for 30 min at 56 °C. Amplification of the D1S80 locus was done using 16 µl of the primary DNA extract. For the AmpF*l*STR Profiler, AmpF*l*STR Blue and genRES MPX kits, 10 µl was used for amplification in a total volume of 25 or 50 µl (according to the manufacturers instructions). Amplification was done using a GeneAmp PCR System 9600 (Perkin Elmer Applied Biosystems, Weiterstadt, Germany). To enhance the sensitivity of AmpF*l*STR Profiler, 1 µl of the primary PCR product was reamplified with 28 cycles, 3 µl of the amplification product was separated by electrophoresis and detected directly using the ABI PRISM 310 Genetic Analyser. Injection times were varied between 5 and 15 s.

Statistical interpretation of the results was done according to Evett and Weir (1998) and two hypotheses have been considered. The prosecution proposition "the crime sample contains DNA from the suspect and the victim" and the defence proposition "the crime sample contains DNA from the suspect and an unknown person". The corresponding probabilities are given as likelihood ratios (LR).

Results and discussion

The results of the analysed loci are summarised in Table 1. Amplification of the D1S80 locus revealed evidence for the presence of DNA from the victim in fingernail debris of the offender's left hand, whereas no signals were detected from debris from the right hand that could be exclusively assigned to the victim.

To obtain more information, three additional loci were analysed with the AmpF*l*STR Blue Kit (D3S1358, vWA, FGA). At all three loci alleles from the victim could be detected but especially in the case of alleles 23 and 26 at the FGA locus, the signal intensity was below 50 relative fluorescent units (RFU, see Table 1). Amplification with the AmpF*l*STR Profiler also showed two additional alleles at the loci D3S1358 (allele 17) and vWA (allele 17), which could be assigned to the victim with signal intensities of 40 and 30 RFU, respectively. All other loci of this multiplex system showed no additional alleles of the victim. To increase the sensitivity of the reaction, reamplification of the primary PCR products was performed. Whereas at the loci D3S1358 and vWA unspecific signals appeared, two additional alleles (alleles 7 and 9) were detectable at the locus TH01. At the gender-specific locus amelogenin, an X:Y ratio of 1.5:1 gave slight indications of the presence of additional female DNA but this could also be due to preferential amplification at this locus (Frégeau et al. 1999). At the loci FGA and TPOX, allelic drop-out occurred. At CSF1PO and D13S317, alleles that could be assigned to the victim were not detectable, probably due to insufficient amplification and/or their appearance in stutter positions of the main DNA component.

For a further increase of the evidential value and to check the sensitivity of the new genRES MPX multiplex kit, PCR was repeated using this kit which contains the loci vWA, ACTBP2 (SE33), TH01, D21S11 and FGA, the core systems of the central genetic database established by the Federal criminal police office of Germany (BKA)

Table 1 PCR results of the fingernail debris of the offender's left and right hand and the reference sample of the victim. In the case of low signal intensity (< 100 RFU), "w" is added to the respective allele designation. Alleles given in *bold letters* represent signals which can be assigned to the victim

System	Locus	Victim	Suspect (right hand)	Suspect (left hand)
D1S80	D1S80	24/30	22/24	22/24/**30w**
AmpF*l*STR Blue Kit	D3S1358	17/19	15/19	15/**17**/19
	vWA	14/17	14/16	14/16/**17**
	FGA	23/26	20/25	20/**23**[a]/25/**26**[a]
AmpF*l*STR Profiler	D3S1358	17/19	15/19	15/**17**[a]/19
	vWA	14/17	14/16	14/16/**17**[a]
	FGA	23/26	20/25	20/25
	Amelogenin	X	XY (1:1)	XY (1.5:1)
	TH01	7/9	6/9.3	6/**7**/**9**/9.3
	TPOX	10/11	9/10	9/10
	CSF1PO	10/12	10/13	10w/13w
	D5S818	11	9/11	9w/11w
	D13S317	8/10	9/11	9w/11w
	D7S820	8/9	8w/12w	No results
genRES MPX	vWA	14/17	14/16	14/16/**17**
	ACTBP2 (SE33)	18/19	21.2/23.2	**18**/**19**/21.2/23.2
	TH01	7/9	6/9.3	6/**7**/**9**/9.3
	D21S11	29/33.2	27/31	27/**29w**/31/**33.2w**
	FGA	23/26	20/25	20/**23**/25/**26**

[a] Signals with intensities below 50 RFU

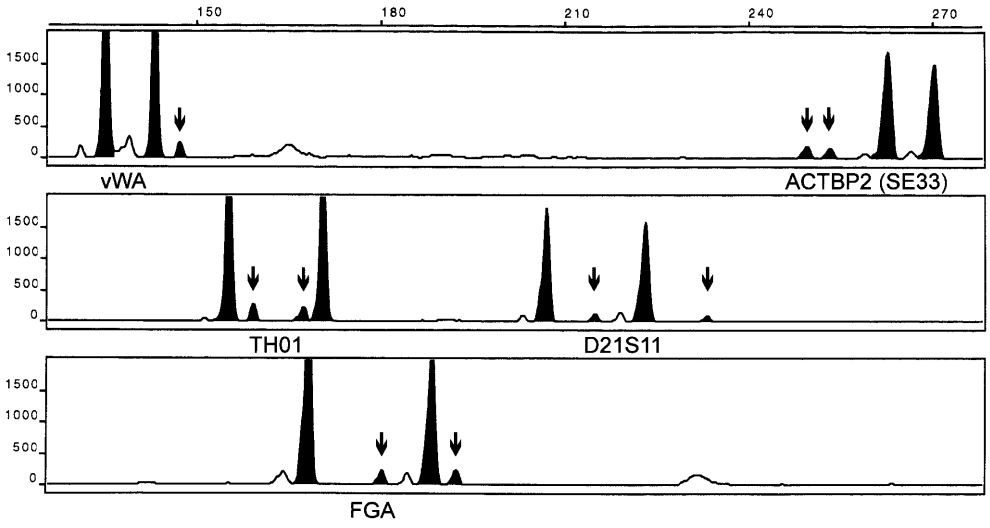

Fig. 1 Electropherogram of genRES MPX amplified DNA from fingernail debris of the offender's left hand (*arrows* depict the alleles which can be assigned to the victim; amplified loci are designated at the bottom of each row)

in 1998. According to the manufacturers information the second generation kit will also include the gender specific amelogenin locus. Alleles specific for the victim could be detected at all loci (Fig. 1) and with intensities of about 100–300 RFU these signals showed about 10% of the intensity of the main signals. Compared to the loci analysed with the AmpF*l*STR kits, the genRES MPX kit includes two highly polymorphic loci (ACTBP2 and D21S11), which contribute to a higher discrimination power (Lederer et al. 2000). This is the reason why in mixed DNA samples, alleles occur less frequently in stutter positions. Although with the genRES MPX kit only five loci were analysed, the likelihood ratio in the current case (2.8×10^8) was about three orders of magnitude higher than the combination of AmpF*l*STR Blue Kit and AmpF*l*STR Profiler (1.4×10^5). In addition, the evidential value of the Blue kit and Profiler results would be further reduced if allelic drop-out would be taken into consideration in the statistical interpretation (Bär and Fukshansky 2000). Although DNA samples had been stored for several months at –20 °C before amplification with the genRES MPX kit, peak heights, especially at the FGA locus were about fourfold higher compared to the AmpF*l*STR Blue kit. This might be due to an overall shortening of the amplification products of this locus in the MPX kit, resulting in less susceptibility for allelic drop-out (Wallin et al. 1998).

The case presented underlines the value of an analysis of fingernail debris despite a longer time lapse and multiple handwashing between the offence and the examination (Wiegand et al. 1993). Even under these circumstances the genRES MPX kit, in contrast to the AmpF*l*STR kits, showed a higher sensitivity and allowed an unequivocal assignment of the biological evidence. It has to be pointed out that fingernail debris should be routinely analysed in violent crimes and sexual assaults although it has been suggested that they no longer need to be sent as items of evidence because of a minimal contribution of evidential value (Hochmeister et al. 1996). Indeed, even in cases where legitimate contact with a possible DNA transfer might occur, the DNA evidence has to be evaluated cautiously and may be used at most as a mosaic piece together with independent evidence. In the presented case, the nurse was not involved in the health treatment of the patient before or after the incident and also made contradictory statements to the police. For example, he was not able to explain why he had sent his female colleagues out of the emergency admission and, consequently, could not invalidate the accusation of having done this to be alone with the patient. Furthermore, the nurse alleged that he had tried to alarm the attendant physician after the incident by beeper, but this was refuted by the physician. The nurse kept contradicting himself; his allegations were judged by the court to be implausible. The DNA analysis was judged to be additional, but weak evidence. Due to numerous independent incriminating facts, the male nurse was sentenced to 9 months imprisonment.

In contrast, DNA from fingernail debris may be of strong evidential value, if any contact between a victim and a suspect is denied whether legitimate or not. Therefore, we recommend that fingernail debris should be recovered in cases of violent crimes, even after a time lapse of some days between incident and examination.

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