Int J Leg Med (1992) 105:59–61

International Journal of
Legal Medicine

Technical notes

Forensic identification of urine samples

B. Brinkmann, S. Rand, and T. Bajanowski

Institut für Rechtsmedizin, Westfälische Wilhelms Universität Münster, Von Esmarch-Strasse 86, W-4400 Münster, Federal Republic of Germany

Summary. VNTR polymorphisms were investigated for the possible individualisation of human urine samples with reference to doping cases in sport. Investigations were carried out with the RFLP single locus system YNH24/Hinf I and the PCR-VNTR systems Apo B and COL2A1 (AMPFLPs) as well as SE 33 and TC 11 (STRs). Urine samples were tested using 3 different volumes (10 ml, 1 ml and 0.1 ml) after 2 days and 2–5 weeks storage at 4°C. Positive results were obtained for STR systems in all cases and with the smallest volume of urine tested (0.1 ml). The AMPFLP systems gave positive results in 4 out of 8 (Apo B) and 5 out of 8 (COL2A1) samples and YNH24 was successful in 1 out of 3 samples. Negative results were obtained for the AMPFLP systems and YNH24 after longer storage periods whereas the STRs were positive.

Key words: DNA polymorphism – Short tandem repeats (STRs) – Urine

Zusammenfassung. Zur Individualisierung menschlicher Urinproben – in Anlehnung an Dopingfälle im Sport – wurden VNTR-DNA-Polymorphismen untersucht. Der Nachweis erfolgte mit dem Single-locus-System YNH24/Hinf I (RFLP-Technik) und den PCR-VNTR's Apo B und COL2A1 (AMPFLP's) sowie SE 33 and TC 11 (STR's). 20 Urinproben (3 unterschiedliche Extraktionsvolumina: 10 ml – 1 ml – 0.1 ml) wurden nach (1) 2 Tagen Lagerung und (2) nach 2–5 wöchiger Lagerung bei 4°C typisiert. Die STR-Systeme führten bei 2 Tage alten Urinproben in allen Fällen zu positiven Ergebnissen (minimales Urinvolumen: 0.1 ml), die AMPFLP's in 4 von 8 (Apo B) bzw. 5 von 8 (COL2A1) Fällen; der Nachweis mit YNH24 gelang in 1 von 3 Fällen. Die mehrwöchig gelagerten Urinproben konnten weder mit YNH24 noch mit AMPFLP's typisiert werden. Mit STR's hingegen verliefen auch diese Nachweisversuche positiv.

Schlüsselwörter: DNA-Polymorphismus – Short tandem repeats (STR's) – Urin

Introduction

In connection with discussions on possible doping cases in sport the question has arisen, whether DNA polymorphisms are applicable to the individualization of human urine samples. A pilot study has been performed to evaluate this problem.

Materials and methods

DNA was extracted in parallel from 1 to 2-day-old blood and urine samples taken from 20 individuals of both sexes. The sediment from the urine samples was extracted using phenoldichloromethane after lysis with proteinase K (Brinkmann et al. 1991). Urine sample sizes of 10, 1 and 0.1 ml were investigated and the amount of extracted DNA was measured fluorimetrically (Sambrook et al. 1989). DNA typing was carried out using one selected single locus system (YNH24/Hinf I, Nakamura et al. 1987) as described previously (Rand et al. 1992a), two selected AMPFLP (amplifiable fragment length polymorphism) systems (Apo B, Boerwinkle et al. 1989; COL2A1, Wu et al. 1990) and two selected STR (short tandem repeat) systems (SE 33, Polymeropoulos et al. 1992; TC 11, Edwards et al. 1991). Further investigations were carried out using the STR systems after storage for 2–5 weeks at +4°C. The investigations for Apo B and COL2A1 were carried out using the method described by Rand et al. (1992b). Amplification and electrophoretic conditions for the STR systems were as follows:
1 ng template DNA; 1 U Taq polymerase (Promega corporation, USA), 0.3 μM each primer, 100 μM of each nucleotide, 5 µl buffer (Promega) diluted to a total volume of 25 µl with distilled water. The reaction mixture was overlayed with 2–3 drops of oil.

SE 33 primer sequence (Polymeropoulos et al. 1992):
5′-AAT CTG GGC GAC AAG AGT GA-3′
5′-ACA TCT CCC CTA CCG CTA TA-3′

TC 11 primer sequence (Edwards et al. 1991):
5′-GTG GGC TGA AAA GCT CCC GAT TAT-3′
5′-ATT CAA AGG GTA TCT GGG CTC TGG-3′

Amplification conditions (B. Budowle personal communication 1992)
SE33: 93°C – 1 min (denaturation), 61°C – 1 min (annealing)
72°C – 1.5 min (extension); 28 cycles
(Thermocycler: Biometra, Triothermoblock, FRG)
TC11: 1) 94°C – 20 s (denaturation), 64°C – 20 s (annealing)
70°C – 60 s (extension); 10 cycles

2) 90°C – 20 s (denaturation), 64°C – 20 s (annealing)
70°C – 60 s (extension); 20 cycles
(Thermocycler: Perkin Elmer Cetus 9600)

Electrophoresis: Polyacrylamide gels (SE 33: 5% T, 3% C – TC 11: 6% T, 3% C – 750 µm, 28 m*M* CHES, 80 m*M* formate, piperazine diacrylamide as cross-linker), 18 cm separation distance; 2% agarose plugs in Tris-borate (0.5 *M* Tris, 0.28 *M* boric acid, pH 9.0) according to Allen et al. (1989). Bands were visualized by silver staining (Budowle et al. 1991).

Results

1. Urine volumes, DNA yield and quality

DNA was extracted from two urine samples using 10, 1 and 0.1 ml urine and from the other urine samples using 10 ml. The extraction from 10 ml urine yielded between 31 and 298 ng/µl DNA from a sediment volume of 50 µl. The total amount of extracted DNA therefore varied between 1.5 and 15 µg.

The DNA extraction from 1 and 0.1 ml urine yielded concentrations which were below the detection limits of the fluorimetric method which is given as 1–5 ng/µl for high molecular weight DNA (Sambrook et al. 1989). The test for high molecular weight DNA using a mini-gel (Rand et al. 1992a) showed in all cases that the DNA was highly degraded.

2. Individualization of urine samples (Table 1)

In all urine samples tested individual band patterns could be demonstrated for the STR systems SE 33 (Fig. 1a) and TC 11 (Fig. 1b), which in each case was identical with the corresponding blood sample. Positive results, which were identical to the corresponding blood samples were obtained for the AMPFLP systems COL2A1 in 5 out of 8 samples and for Apo B in 4 out of 8 samples. Negative results were obtained in all other cases.

An individual band pattern could only be demonstrated in 1 out of 3 investigated samples using the single locus system YNH24.

Reliable and reproducible results were obtained for the STR system SE 33, even with 1 and 0.1 ml urine although the amount of extracted DNA was less than 5 ng/µl.

Table 1. Relative efficiency of DNA analysis in urine samples using different approaches. n.t. = not tested

	YNH24	AMPFLP		STR	
		COL2A1	Apo B	SE 33	TC11
Urine (2 days old)	33%	62%	50%	100%	100%
Urine (5 weeks old)	0	0	0	100%	n.t.

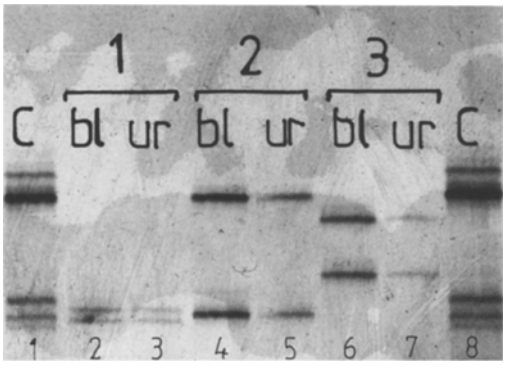
3. Storage of urine samples

After storage of urine samples for 2–5 weeks at 4°C, sufficient DNA could be extracted for amplification from the sediment of 20 ml of urine. DNA typing was carried out on two samples using TC 11 and SE 33, and in each case the results corresponded with those obtained from the blood sample. Negative results were obtained in each case using both AMPFLP systems and the single locus probe YNH24.

Discussion

As demonstrated in this pilot study, genetically determined DNA polymorphisms can be identified in urine samples, but the success rate seems to be closely correlated to the method of DNA investigation. Positive results can normally be obtained using STRs, frequently with the AMPFLP systems but only occasionally with the relatively sensitive single locus system YNH24.

The increase in sensitivity achieved by the various DNA technologies has been previously reported by this group (Brinkmann 1992). By experimental studies on DNA extracted from blood samples an increase in sensitivity of roughly 4 degrees of magnitude could be demonstrated between multi-locus systems and STRs, which has been confirmed by this study. However, Roewer et



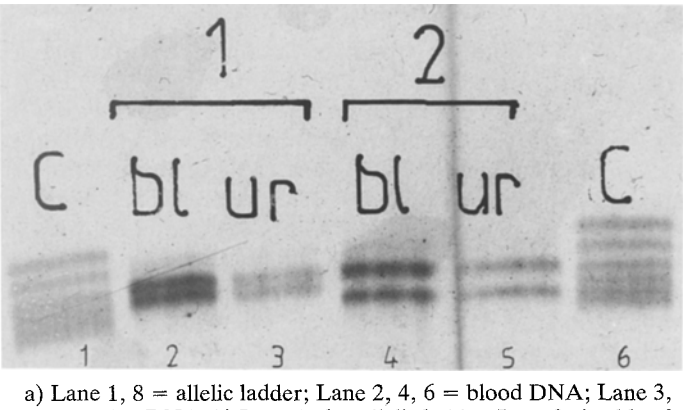


Fig. 1. Comparision between paired blood and urine amplification patterns (DNA isolation from 0.1 ml urine) from unrelated individuals using the STR system SE 33 (a) and TC 11 (b) (for amplification and electrophoresis conditions see Materials and methods). a) Lane 1, 8 = allelic ladder; Lane 2, 4, 6 = blood DNA; Lane 3, 5, 7 = urine DNA; b) Lane 1, 6 = allelic ladder; Lane 2, 4 = blood DNA; Lane 3, 5 = urine DNA

al. (1990) reported that 200 ml of urine was necessary to produce a DNA fingerprint. The urine volume could be reduced to 0.1 ml in the present study. In practical cases therefore a volume of 10 ml fresh urine is sufficient for investigations in several systems including the necessary controls and repeat investigations.

As is well known, the various systems applied here show very different sensitivities to degradation. Using multi-locus and single locus systems (restriction fragment length polymorphisms = RFLP's) high molecular weight DNA in the range of up to 30 Kb (1 Kb = 1000 basepairs) is normally necessary. By the use of AMPFLPs fragment lengths of approx. 1000 bp are normally sufficient to obtain adequate results and using STRs the fragment length range can be reduced by approximately one-third (ca. 300 bp). This signifies that STRs are extremely insensitive to degradation. The application of these systems is therefore extremely promising in this context, because in urine samples the degree of degradation is extremely high due to bacterial contamination. This was also confirmed by the present study. Degradation due to storage (up to 5 weeks at 4°C) therefore seems to have no influence on the results.

A comparison of the DNA pattern from urine samples with other samples of human origin would normally be carried out using a blood sample, which is this respect is unambiguous. If legal considerations oppose the taking of a blood sample, other control samples are, as demonstrated here, just as suitable (e.g. saliva samples, oral swabs and plucked hairs). Using the STR systems identifiable band patterns can be routinely and clearly detected from these materials (Brinkmann 1992).

Two possibilities exist for the evaluation of comparative investigations (comparison between DNA from urine and human control DNA):

(a) The individual band patterns do not match, i.e. samples cannot come from the same person (exclusion).
(b) The band patterns from the urine and control sample match; i.e. the samples can originate from the same person (non-exclusion).

In the case of a match a statement on the frequency of the corresponding band pattern is normally necessary. Depending on whether 2 or 3 systems were applied, the 20 urine samples investigated in this study gave individual band pattern frequencies between 1:1600 and 1:40000. The inclusion of 5 such STRs (e.g. SE 33 – Polymeropoulos et al. 1992, TC 11, TC 6 – Edwards et al. 1991, vWa – (P. Gill personal communication 1992), MBP – (B. Budowle personal communication 1992) would routinely lead to frequencies of 1:1 million or greater.

The typing of urine samples using DNA technology can therefore be applied within the scope of doping investigations to prove identity or to furnish proof of non-identity.

It must be emphasized, that such tests must be forensically validated, because the possibility of artefacts does exist. These must be absolutely avoided by careful observation of an extensive repertoire of controls and corresponding safety measures. If the samples are stored at 4°C a time delay of several days between sampling and investigation can be tolerated. DNA band patterns from a single individual which have been previously determined remain stable. Succeeding investigations on urine samples therefore need only a single investigation of the control sample (e.g. blood, saliva, hair). The polymorphisms shown here are exclusively VNTR polymorphisms and therefore refer to non-coding DNA sequences. Inferences on personal traits are therefore not possible by such investigations.

Acknowledgement. The authors would like to thank M. Schürenkamp for excellent technical assistance.

References

Allen RC, Graves G, Budowle B (1989) Polymerase chain reaction amplification products separated on rehydratable polyacrylamide gels and stained with silver. BioTechniques 7:736–744

Boerwinkle E, Xiong W, Fourest E, Chan L (1989) Rapid typing of tandemly repeated hypervariable loci by the polymerase chain reaction: application to the apolipoprotein B 3′ hypervariable region. Proc Natl Acad Sci USA 86:212–216

Brinkmann B, Rand S, Wiegand P (1991) Population and family data of RFLP's using selected single- and multi-locus systems. Int J Leg Med 104:81–86

Brinkmann B (1992) The use of STRs in stain analysis. In: Proceedings from the third international symposium on human identification, Promega Corporation, Madison, USA (in press)

Budowle B, Chakraborty R, Giusti AM, Eisenberg AJ, Allen RC (1991) Analysis of the variable number of tandem repeats locus D1S80 by the polymerase chain reaction followed by high resolution polyacrylamide gel electrophoresis. Am J Hum Genet 48:137–144

Edwards A, Civitello A, Hammond HA, Caskey CT (1991) DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. Am J Hum Genet 49:746–756

Nakamura Y, Leppert M, O'Connell P, Roger W, Holm T, Culver M, Martin C, Fujimoto E, Hoff M, Kumlin E, White R (1987) Variable number of tandem repeat (VNTR) markers for human gene mapping. Science 235:1616–1621

Polymeropoulos MH, Rath DS, Xiao H, Merril CR (1992) Tetranucleotide repeat polymorphism at the human beta-actin related pseudogene H-beta-Ac-psi-2 (ACTBP2) Nucleic Acids Res 20(6):1432

Rand S, Wiegand P, Brinkmann B (1992a) Problems associated with the DNA analysis of stains. Int J Leg Med 104:293–297

Rand S, Puers C, Skowasch K, Wiegand P, Budowle B, Brinkmann B (1992b) Population genetics and forensic efficiency data of 4 AMPFLPs. Int J Leg Med 104:329–333

Roewer L, Nürnberg P, Fuhrmann E, Rose M, Prokop O, Epplen JT (1990) Stain analysis using oligonucleotide probes specific for simple repetitive DNA sequences. Forensic Sci Int 47:59–70

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning. A new laboratory manual. Cold Spring Harbour Press, Cold Spring Harbour New York

Wu S, Seino S, Bell GI (1990) Human collagen, type II, alpha 1, (COL2A1) gene: VNTR polymorphism detected by gene amplification. Nucleic Acids Res 18:3102