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FORENSIC APPLICATION OF TWO SHORT TANDEM REPEAT SYSTEMS: HUMTH01 AND HUMvWA31.

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INTRODUCTION

Over the past few years many laboratories worldwide have introduced analysis of DNA polymorphisms as a routine method for forensic purposes. In particular, VNTRs, investigated by PCR, have reached an important position after considerable development. At present laboratories are studying a further series of highly polymorphic loci whose polymorphism derives from a dimeric-trimeric-tetrameric tandem repeated core sequence, named short tandem repeats (STRs) (Weber and May 1989; Edwards et al.1991). The aim of the present study was to investigate two of these STRs, HUMTH01 (Edwards et al. 1992) and HUMvWA31 (Kimpton et al. 1992) to evaluate:1) allele frequencies in an Italian population sample, and 2) their efficiency for personal identification on aged bloodstains from various substrates.

MATERIALS AND METHODS

Stain preparation : a series of stains prepared from 3, 6,10 and 20 µl of whole fresh blood obtained from six subjects was collected on paper, white cotton and glass and were stored for 3 years at room temperature.
DNA extraction: extracted from the fresh blood of unrelated healthy subjects (HUMTHO1 n= 288; HUMvWA31 n=211) and from 72 bloodstains using phenol-chloroform (Budowle and Baetchel 1990) and chelex (Walsh el al. 1991) methods.
PCR amplification: according to Brinkmann et al. (1993a) for HUMTH01 and Wiegand et al. (1993) for HUMvWA31 with the primers proposed by Edwards et al. (1991) for HUMTH01 and by Kimpton et al. (1992) for HUMvWA31.
Electrophoresis: separation by high-resolution PAGE (Budowle et al. 1991) for HUMTH01 and denaturing PAGE for HUMvWA31 using a discontinuous buffer (Allen et al. 1989). Bands were visualised by silver staining (Budowle et al. 1991) and phenotyped by side-to-side comparison with an allelic ladder (Fig. 1 a,b,c).

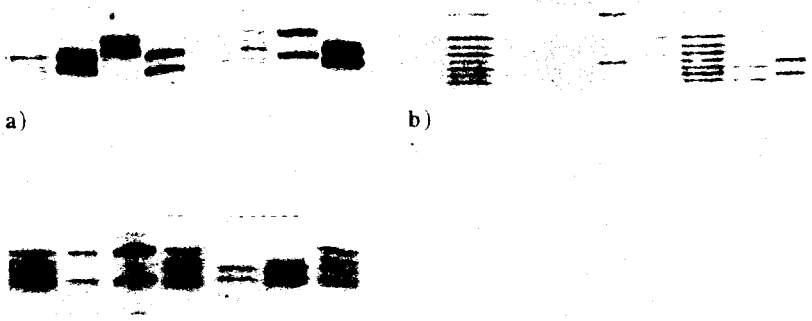

Fig. 1 (a,b,c) Amplified DNA fragments in HUMTH01 and HUMvWA31 systems using polyacrylamide gels and silver staining.
a) HUMTH01: from left to right: allele ladder is in lanes 1,5,6: HUMTH01 phenotypes: lane 2: 8-9.3; lane 3: 6-7; lane 4: 8-10; lane 7: 6-9; lane 8: 8-9.3.
b) HUMvWA31: from left to right: allele ladder is in lanes 1,6; HUMvWA31 phenotypes: lane 2: 17-18; lane 3: 15-19; lane 4: 11-17; lane 5: 14-16; lane 7: 18-20; lane 8: 17-19.
c) HUMTH01: from left to right: allele ladder is in lanes 1,4,7: lanes 2 and 3: 6-9.3 phenotypes from 3 and 20 µl aged bloodstains; lanes 5, 6: 8-9.3 phenotypes from 3 and 20 µl aged bloodstains.

RESULTS AND DISCUSSION

Six alleles for HUMTH01 (183-207 bp) and 7 for HUMvWA31 (138-162 bp) were observed. A new allele located on the anodic side of allele HUMvWA31*14 was found (temporarily designated as HUMvWA*11). The frequency distribution of HUMTH01 and HUMvWA31 alleles is shown in figure 2 a,b. Observed and expected genotypes were compared by assuming the Hardy-Weinberg law (HUMTH01 χ^2 = 9,40 0.80< P <0.90; 15 d.f.- HUMvWA31 χ^2= 22.166 0.75< P <0.90; 28 d.f.). The power of discrimination (PD) and chance of exclusion were, respectively, 0.92 and 0.57 for HUMTH01 and 0.93 and 0.61 for HUMVWA31, suggesting that these STR systems may be a powerful tool in forensics. Table 1 summarizes the results expressed as percentages of amplification and typing from aliquoted bloodstains on various substrates, using two extraction methods (phenol-chloroform vs chelex). The results show that: a) 3 µl are sufficient to obtain positive amplification; b) paper and glass are better substrates than white cotton; c) in our bloodstain storage conditions, phenol-isoamyl alcohol was better than the chelex method.

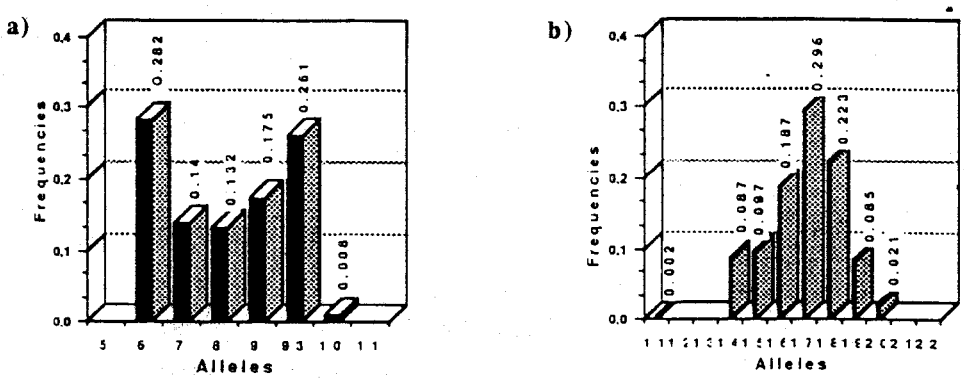

Fig. 2 (a,b) Frequency distribution of HUMTH01 (a) and HUMvWA31(b) system alleles in an Italian population sample.

	PAPER		WHITE COTTON		GLASS	
SIZE	HUMTH01 % a b	HUMvWA31 % a b	HUMTH01 % a b	HUMvWA31 % a b	HUMTH01 % a b	HUMvWA31 % a b
20 mcl	100 (33.3)	100 (16.7)	16.7 (66.7)	66.7 (66.7)	100 (66.7)	100 (33.3)
10 mcl	100 (33.3)	100 (16.7)	16.7 (66.7)	50 (50)	100 (66.7)	100 (33.3)
6 mcl	100 (33.3)	83.3 (16.7)	16.7 (66.7)	50 (50)	83.3 (50)	83.3 (16.7)
3 mcl	83.3 (33.3)	66.7 (16.7)	83.3 (50)	16.7 (16.7)	66.7 (33.3)	83.3 (16.7)

a) phenol-chloroform; b) chelex

Table 1. Amplification and typing of HUMTH01 and HUMVWA 31 from bloodstains of different sizes on various substrates using two extraction methods

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