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The validation of a 7-locus multiplex STR test for use in forensic casework

(I) Mixtures, ageing, degradation and species studies

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Abstract We have evaluated a multiplex STR system for routine forensic use, which co-amplifies six short tandem repeat (STR) loci; HUMTH01, D21S11, D18S51, D8S1179, HUMVWF31/A and HUMFIBRA (FGA), in conjunction with the X-Y homologous gene Amelogenin. Analysis of PCR products employs denaturing polyacrylamide gels coupled with fluorescentlabelled primers and detection is undertaken on ABD 373A automated sequencers. The technique was shown to be robust and reproducible when samples were analysed under conditions consistent with those encountered in a forensic environment.

The system was demonstrated to be human specific and is suitable for use with both aged and degraded material. Somatic stability was proven with a wide range of tissue types and we were able to detect mixtures at ratios between 1:10 and 10:1. During this study no incidence of sample mis-typing due to allelic or locus drop-out was observed. Furthermore, although additional artefact bands were occasionally encountered these did not interfere with the interpretation of results. The performance of the system with poor quality samples demonstrated its suitability as a powerful tool in forensic investigation.

Key words STR's · Multiplex amplification · Forensic identification

Introduction

The analysis of short tandem repeat (STR) loci by DNA amplification or polymerase chain reaction (PCR) is currently the method of choice for use in routine forensic investigations. The technique is particularly useful in the analysis of highly degraded samples or minute stains, [1–6] where the initial quantity or quality of DNA is unsuitable for other DNA profiling methods.

An abundance of STR loci in the human genome has resulted in a wide choice of loci [7, 8], however selection for their use in forensic casework usually depends on discriminating power, their ability to co-amplify with other loci in a multiplex reaction [9–11] and their structure. STR loci can be defined as simple, compound or complex depending on the uniformity of their repeat sequence [12]. The STRs studied in this paper are three simple and three complex tetranucleotide loci combined with the amelogenin sex test [11, 13] these form the Second Generation Multiplex System (SGM). This system is highly discriminating displaying a probability of chance association of 1×10^{-8}, roughly equivalent to four single locus probe (SLP) tests. The physical properties and parameters of the system are described by Kimpton et al. [14]. Automated sequencers and specialist software allow the precise sizing of products, detected using fluorescent dye markers, on polyacrylamide gels [10, 15–18], thus eliminating the need for continuous allele distribution models.

Multiplex reactions are a compromise of ideal conditions and loss of efficiency of amplification may result. The main reason for using multiplexes is to speed the process. This must be viewed in the context of recent legislation in the United Kingdom which has initiated the formation of a national DNA database with an intended throughput of c.135,000 samples per annum. However, the use of multiplex and singleplex systems are not mutually exclusive. Singleplex reactions may occasionally be required to clarify or enhance a difficult result.

Validation of the SGM was designed in consultation with the guidelines recommended by the DNA commission of the International Society of Forensic Haemogenetics [19] for the validation of STRs. In this paper the following were investigated: analysis of old and degraded material, somatic stability, species specificity, mixtures. Subsequent communications will examine the following; analysis of simulated and actual cases, interpretation of results, allele designation [20, 21].

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Materials and methods

Preparation of samples

Blood, semen and saliva stains were prepared on boiled cotton from fresh liquid samples, these were then air dried before storage at –20° C.

Species

DNA was prepared as above and true concentrations of non-human DNA were calculated by running 1 µl of extracted DNA on an agarose gel and comparing this to a sample of known concentration.

DNA from the Gorilla, Chimpanzee, Owl monkey, Green monkey and Galago was a kind donation from Mark Batzer of the Laurence Livermore Institute, USA.

Blood from the Spider monkey was a kind donation from Dudley Zoo, England.

Blood from the Black ape and Entellus langur was a kind donation from Dr J. Kirwood, Department of Veterinary Science, Institute of Zoology, Regents Park, London.

DNA from the Japanese non-primates was a kind donation from Dr E. Naito, Department of Forensic Medicine, Niigata University School of Medicine, Japan.

Blood from the Elephant was a kind donation from Chester Zoo, Wales.

Blood from the Frog and Toad were a kind donation from Paul Townsend, Biomedical Services, Birmingham University.

Bacterial DNA was either a kind donation from the European Collection of Animal Cell Cultures, Centre for Applied Microbiology & Research, Salisbury, Wiltshire or supplied by the Public Health Laboratory Service, National Collection of Type Cultures, 61 Colindale Avenue, London

Ageing studies

Four different sets of ageing studies have been carried out, all stains were stored under normal humidity conditions, at room temperature and were prepared as above.

1) 48 Blood stains prepared at different times since 1982 (13 years oldest stain)
2) 12 Semen stains stored for 6 months plus one stain 13 years old.
3) 16 Saliva stains stored for 6 months (examined in direct comparison to the 12 semen stains listed above).
4) 23 Saliva stains stored for 15 months (repeat of stains tested above) plus 2 stains 10 years old.

Degradation studies

Hair, blood, semen and saliva stains were prepared as above and degraded at 37° C in a humid box for the designated time period (between 0 and 90 days).

Mixture experiments

Three different mixture experiments were set up. Six combinations of liquid blood were mixed volumetrically in proportions of 1 : 1, 1 : 2, 1 : 5, 1 : 10, 10 : 1, 5 : 1, 2 : 1 and extracted using the Chelex method; 1 and 5ng of each combination were amplified and typed as above. The same allele combinations were used and mixed in the same proportions for the DNA:DNA mixtures. These were made up from liquid bloods, extracted as above and quantified several times to ensure accuracy of measurement. Liquid semen and saliva were mixed and prepared in the same proportions and manner as the liquid bloods.

Preparation of DNA

DNA was prepared from samples using a rapid Chelex extraction [22]; for non-human DNA a phenol-chloroform extraction was used followed by quantitation using spectrophotometry. All other DNA samples were quantified by a dot-blot system detailed by Walsh et al. [23] which uses a biotinylated probe complementary to a primate-specific alpha satellite DNA sequence (D17Z1) on chromosome 17 [24].

Amplification conditions

The repeat unit and primer sequences for the STR loci have been described previously [11]. Human DNA (100pg-5ng) or non-human DNA (100pg-50ng) was amplified in a total reaction volume of 50 µl. This consisted of 1 X PARR buffer (10 m*M* Tris-HCL pH 8.3, 50 m*M* KCL, 1.5 m*M* $MgCl_2$ 1% Triton-X-100 – Cambio Laboratories, England), 1.25 units Taq polymerase, 200 µM of each dNTP, and primers Amelogenin 1/2, HUMTH01 1/2, D21S11 1/2, D18S51 1/2, D8S1179 1/2, HUMVWF31/A 1/2 and HUMFIBRA 1/2. This was then sealed with one drop of mineral oil. Amplification was carried out in a 9600 thermal cycler (Perkin Elmer) using the following conditions. 93° C for 30 s, 58° C for 75 s: 72° C for 15 s, for 30 cycles followed by a 10 min extension at 72° C.

Detection system

Amplification reaction mixtures (1–2 µl) were combined with 6fmol of an internal lane size standard (GS2500, GS500 or GS350) supplied by Applied Biosystems division (ABD). These were heat denatured prior to loading onto a 6% polyacrylamide denaturing sequencing gel as described by Kimpton et al. [10]. Gels were electrophoresed for 5–6 h at constant power (38 or 30W) on an Applied Biosystems automated DNA sequencer model 373A. Fragment sizes were determined automatically using Genescan 672 software (ABD) employing the local Southern method [25].

Nomenclature

Nomenclature follows the recommendations of the DNA commission of the International Society of Forensic Haemogenetics [19] with the exception of D21S11 [12]. Alleles are designated according to the number of repeat units present. If a repeat is incomplete then the allele is designated by the number of complete repeats present followed by a decimal point, then the number of bases of the incomplete repeat. For example the HUMTH01 9.3 allele contains 9 tetrameric repeats plus an additional repeat consisting of 3 bases. In our laboratory the HUMTH01 9.3 and 10 alleles which differ by 1 base pair are currently combined and designated 9.3.

Results

Five different studies are reported in this paper which examine the reliability of the SGM under conditions routinely encountered in forensic casework; somatic stability, species studies, ageing and degradation studies and mixtures. The original SGM system was initially designed to incorporate seven loci and a sex test, however during the course of the validation one of the loci – D20S85 was removed because the primer sequences generated an excessive number of artefact bands. Removal of this locus from the multiplex had no significant effect on the characteristics of the remaining loci [14].

Somatic Studies

Liquid blood and saliva, blood and saliva stains, hair roots, spermic and aspermic semen, and vaginal swabs

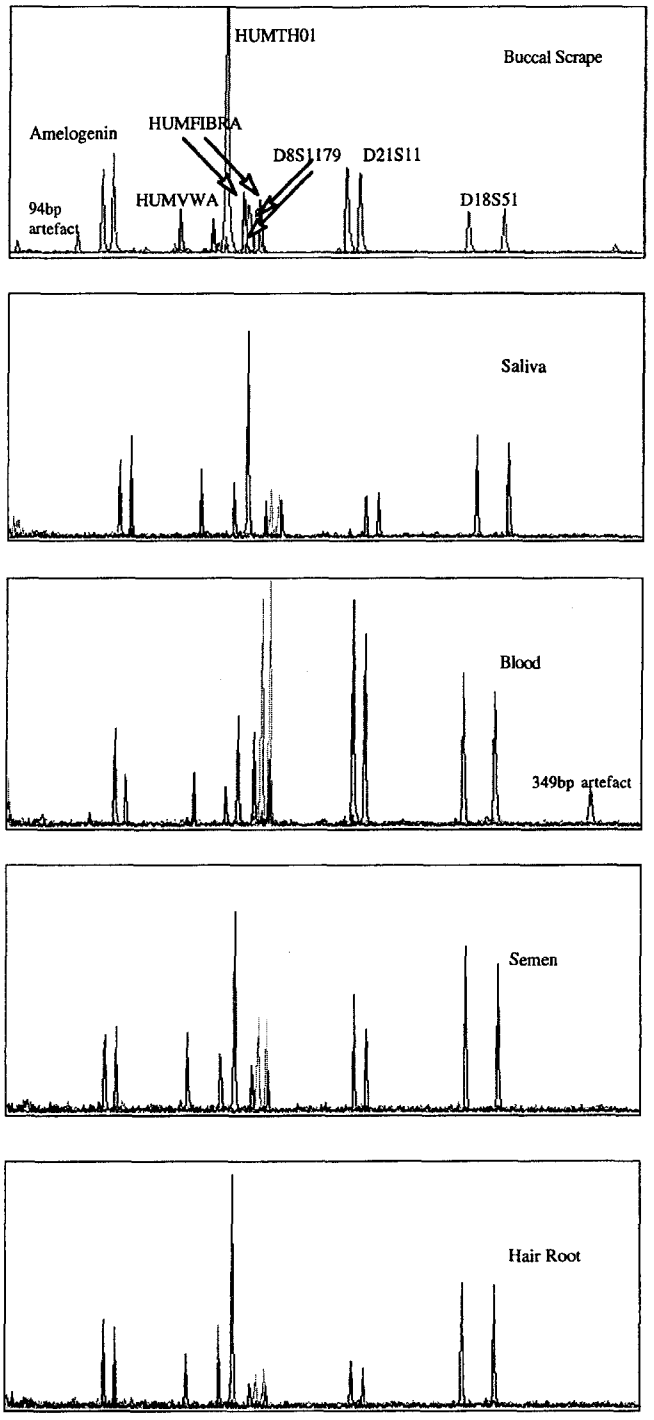

Fig. 1 The results obtained from matched sets of PCRs demonstrate the profiles produced when different sample types from the same individual were extracted and amplified. The vertical scale of each profile is consistent at 0–2000 units which represents peak height. Although each sample gave the same genotype the relative peak areas of the individual loci vary for each tissue and body fluid type. For example the amplification of D21S11 was enhanced in the blood sample compared to the saliva sample

(semen free) from a total of 25 individuals were analysed in order to demonstrate somatic stability. For any given donor all the possible tissue types tested gave the same genotype but the performance of each individual locus varied (Fig. 1). However, the general profile morphology was reproducible between samples of the same body fluid from different donors (Fig. 2). This has consequences for the design and quality testing of multimix for use with varying sample types.

Buccal scrapes, used to sample epithelial cells from inside the cheek, were selected for use in the national DNA database due to their non-invasive nature and the high yield of DNA obtained. Experimentation revealed profiles with a stronger signal but similar morphology to the saliva samples (Fig. 1).

Increased artefact activity, particularly near the Amelogenin and HUMTH01 read regions, in conjunction with a high level of background, was detected in the aspermic semen, vaginal swabs and saliva samples. On average, these samples also gave the lowest signals. Profiles obtained from semen were of the highest quality, with intense even signals displaying very few non-allelic peaks. No allelic drop out was seen.

Species testing

Bacteria. It is standard for 1–5ng human DNA to be analysed. To exaggerate any possible effect, approximately 50ng of chromosomal DNA from each of 26 bacterial strains (Table 1) was analysed by alpha-satellite quantitation and SGM amplification. All strains failed to yield detectable quantitation signals with the alpha-satellite probe and 16 out of 26 strains also failed to produce any detectable amplification products (Table 1). Five strains produced a number of minor peaks within allele ranges, although all of these were below our standard cut-off value. At such high DNA levels a degree of non-specific amplification is not unexpected in the absence of specific target DNA. The quantity of bacterial DNA required to produce any detectable amplification products would not be expected under normal caseworking conditions where less than 5ng of human DNA would be routinely analysed.

Non-primate DNA. Alpha-satellite quantitation signals for all non-primate species tested were at least 100-fold less than their true values (Table 2). Some non-specific amplification products were observed at high DNA levels (100ng – Table 2). In particular a blue peak sizing at approximately 104 base pairs was observed in several of the non-primate species. Although produced by the amelogenin primers [26] the product is smaller than the products from the X or Y chromosomes found in primates and there is also no sex dependant pattern. With the exception of this band, these non-specific amplification products are unlikely to occur with a human/non-human mixture given the normal quantity of DNA amplified under routine casework conditions.

Primate DNA. Alpha-satellite quantitation signals varying in intensity, were obtained for each of the 10 primate species tested (Table 3). As the relationship to humans increases there appears to be an increase in the number of monomorphic and even polymorphic loci detected. How-

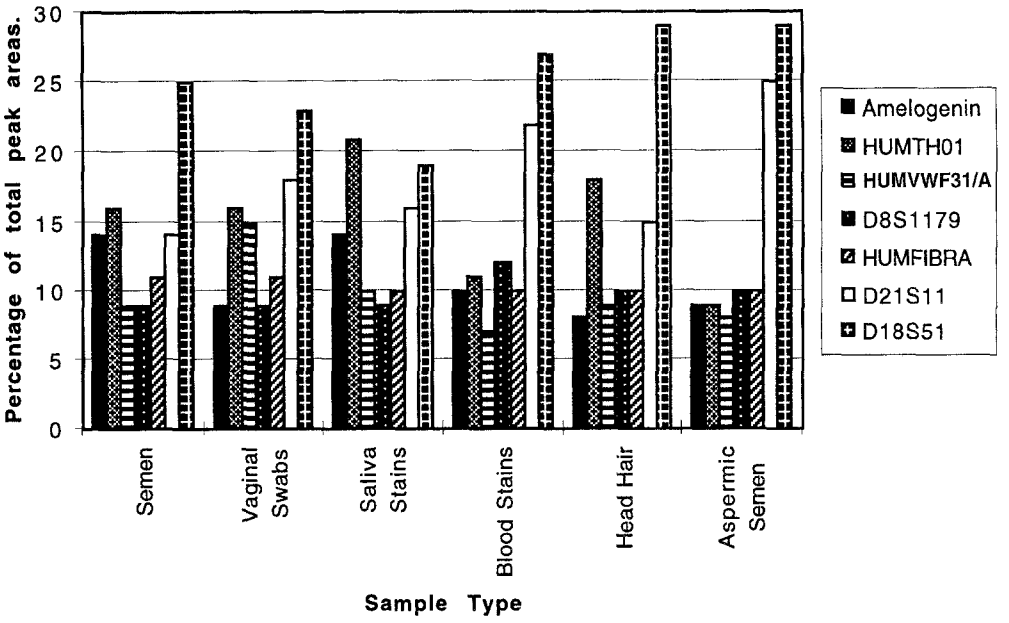

Fig. 2 The results obtained from the somatic study demonstrate that although relative peak areas may differ for each sample type, the pattern or profile array of each sample type is reproducible. A total of 10 females and 15 males (10 providing spermic and 5 providing aspermic semen) were included in this study

Table 1 A summary of the peaks observed with bacterial DNA. All of the gels were sized with GS500. The size of each peak is approximate and where possible has been allocated to the nearest common allele in a human locus read region. Column 9 lists the peaks which could not be attributed to the range of a human SGM locus. The peaks shown in brackets were observed as minor peaks within the profiles (i.e. peak height between 50 and 100 units).

Species	Amelogenin	HUMTH01	D21S11	D18S51	D8S1179	HUMVWF31/A	HUMFIBRA	Out of locus range
Bacillus cereus								
Bacillus thuringiensis								
Bacillus mycoides								
Bacillus anthracis								
Bacillus stearothermo.								
Clostridium botulinum A								
Clostridium botulinum B			(221 – 61)					
Clostridium botulinum B			(230 – 65)					
Clostridium botulinum G								
Clostridium butyricum								
Clostridium difficile								Blue – 101
Clostridium serogenes	108 – X							
Clostridium barati F								Blue – 153 Green – 236
Clostridium pentningenus								Blue – 101, (124)
Clostridium therniocellum								Blue – 130
Clostridium acetobutylicum								
Pseudomonas fluorescen				(295 – 15)				Blue – 259 (342)
Pseudomonas fluorescen				(328 – 32)				
Escherichia coli B								
Streptococcus aummo								
Staphylococcus aureus								
Staphylococcus epidermis								
Candida albicans								Blue – 113, 121, 130
Treponema								Blue – 89, 95, 99
Neisseria gonorrhoeae								
Actinomyces israelii								
Lactobacillus odontolyticus								
Salmonella enterica								Blue – 121, 135, (158, 264)

Table 2 A summary of the peaks observed with non-primate DNA. The alpha-satellite quantitation signals are listed in column 3, column 2 shows the quantity of DNA loaded on the quantitation membrane that gave the result shown. All of the gels were sized with GS500. The size of each peak is approximate and where possible has been allocated to the nearest common allele in a human locus read region. Column 11 lists the peaks which could not be attributed to the range of a human SGM locus. The peaks shown in brackets were observed as the minor component in the profiles. Several of the species were not quantified, these are listed as ND

Species	DNA loaded – ng	Quant Result – ng	Amelogenin	HUMTH01	D21S11	D18S51	D8S1179	HUMVWF31/A	HUMFIBRA	Out of locus range
Fish										
Koi carp *(Cyprinus carpio)*										
Herring *(Clupea harengus)*	50	0								
Sea trout *(Salmo trutta)*	50	0			207 – 54					
Amphibians										
Frog *(Rana temporaria)*	50	0	(112 – Y)							(Blue – 140)
Toad *(Xenopus laevis)*	100	0.08								
Birds										
Pigeon *(Columba livia)*	100	0.1								Yellow – 103
Chicken (UK) 2 tested *(Gallus gallus)*	50	0		174 – 9.3						Blue – 202
Chicken (Japanese) *(Gallus gallus)*	ND	ND								
Duck *(Anas platyrhynchos)*	50	0		156 – 5						
Turkey *(Meleagris gallopavo)*	50	0								
Goose *(Anser anser)*	50	0.5								
Pheasant *(Phasianus colchicus)*	50	0							189 – 12	Blue – 259
Mammals										
Mouse (Japanese) *(Mus musculus)*	50	0								
Rat (UK) *(Rattus rattus)*	50	0								
Rat (Japanese) *(Rattus rattus)*	ND	ND								
Guinea pig *(Cavia cutleri)*	50	0		158 – 6				(158 – 18.2) (162 – 19.2)		(Blue – 194) (Yellow – 166)
Rabbit *(Oryctolagus cuniculus)*	50	0		163 – 7	213 – 57					Blue – 101
Cat *(Felis catus)*	100	0.1								
Dog *(Canis familiaris)*	50	0				(280 – 7)		(200 – 19)		Blue – 104
Fox *(Canis vulpus)*	100	0.1								Blue – 104
Badger *(Meles meles)*	ND	ND								Blue – 102, 106
Lamb *(Ovis aries)*	100	0.5						164 – 20		Blue – 104
Sheep *(Ovis aries)*	ND	ND	(108 – X)	(162 – 7) (170 – 9)						
Pig (UK) *(Sus scrofa)*	ND	ND			(214 – 57)					Blue – 101, 105
Pig (Japanese) *(Sus scrofa)*	ND	ND								Blue – 104

Table 2 (continued)

Species	DNA loaded – ng	Quant Result – ng	Ame-logenin	HUMTH01	D21S11	D18S51	D8S1179	HUMVWF31/A	HUMFIBRA	Out of locus range
Sika deer (Cervus sika)	100	0.09								
Red deer (Cervus elaphus)	100	0.09								
Cow (Bos taurus)	100	0.4								Blue – 104
Horse (Equus caballus)	50	0			(208 – 55)					Blue – 104
Elephant (Loxodonta africana)	50	0								

Table 3 A summary of the peaks observed with primate DNA. All of the gels were sized with GS500. The size of each peak is approximate and where possible has been allocated to the nearest common allele in a human locus read region. Column 11 lists peaks which could not be attributed to the range of a human SGM locus. If confirmed, the sex of the animal has been listed. Peaks shown in brackets were considerably smaller than other peaks observed in that locus read range

Species	DNA loaded – ng	Quant Result – ng	Ame-logenin	HUMTH01	D21S11	D18S51	D8S1179	HUMVWF31/A	HUMFIBRA	Out of locus range
Great Apes										
Chimpanzee – 2 tested 1 *(Pan troglodytes)*	1	1.5	108 – X	159 – 6		170 – 10			187 – 10	Blue – 183, 187
			114 – Y			174 – 11			191 – 13	Yellow – 125, 129, 133
	2	1	1.5	108 – X	160 – 6		170 – 10			179 – 6	Blue – 199, 203
				114 – Y	163 – 7		178 – 12			202 – 18	Yellow – 127, 130, 133
Gorilla *(Gorilla gorilla)*	100	5	108 – X	163 – 7	253 – 77		174 – 11	156 – 18	218 – 26	Blue – 105, 143, 151
			(113 – Y)		257 – 79		182 – 13			Yellow – 236
Orangutan *(Pongo pygmaeus)*	1	4.5	108 – X		248 – 74		186 – 14		205 – 24.2	Blue – 99,127,136,151
							190 – 15			Yellow – 113, (179, 187)
Old World Monkeys										
African green monkey *(Cercopithecus sabaeus)*	100	0.1	108 – X			317 – 20.2				
Japanese monkey *(Macaca fuscata)*	50	5	108 – X	163 – 7	251 – 76 255 – 78			166 – 20.2	219 – 27	Blue – 145
Entellus langur (female) *(Presbytis entellus)*	50	0.5	108 – X		251 – 76			137 – 13 147 – 15.2	190 – 20.2	Blue – 100 Yellow – 161,173,189
Celebes black ape (male) *(Cynopithecus niger)*	50	5	108 – X					162 – 19.2 171 – 22	193 – 14 216 – 25	Blue – 100,131,146,258
										Yellow – 114
New World Monkeys										
Spider monkey (female) *(Ateles sp)*	100	4	108 – X		215 – 58					(Blue – 341)
Owl monkey *(Aotes trivirgatus)*	100	0.1	108 – X		230 – 65					Blue – 101
Prosimians										
Greater bush baby *(Galago crassicaudatus)*	100	0.1								

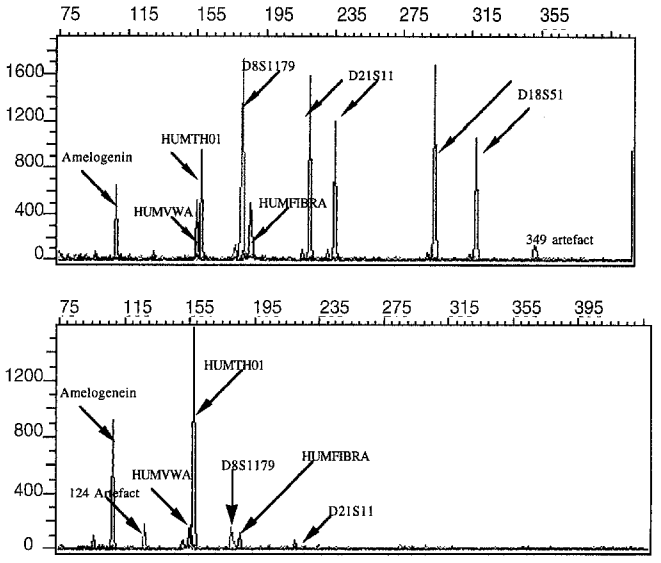

Fig. 3 A, B As samples degrade the high molecular weight alleles tend to drop off first. As a result preferential amplification of the low molecular weight loci may occur; Amelogenin and HUMTH01 in particular give relatively stronger signals as time progresses. The size of the products in bases is shown along the γ axis and the χ axis shows the peak height in arbitrary units. **A** Profile from fresh blood stain, **B** degraded profile from blood stain-day 50

ever, although polymorphic loci were observed when tested with the SGM they were atypical: allele peaks were present both within and outside human locus read regions, no "full" profiles were obtained that included peaks within the range of every locus, and multiple signals observed in read regions were not of similar peak area i.e. the profiles appeared to be unbalanced.

Degradation

Of the four sample types tested the saliva stains showed the greatest degree of degradation. As the time study progressed allele signals gradually decreased with the exception of the low molecular weight loci. Amelogenin and HUMTH01, in particular, demonstrated locus preferential amplification and gave relatively stronger signals (Fig. 3). This pattern was commonly seen with all degraded material until all loci failed to produce a signal. No full profiles were obtained from saliva samples after a maximum of 17 days. Blood and semen stains were generally much more resilient – some individuals gave full profiles after 60 days, although signs of degradation were usually observed. Hair roots gave full, even profiles up to and beyond 90 days and in general showed very little signs of degradation.

During the degradation study no mis-typing of samples occurred, artefacts were rarely observed and no bands were present in locus-read regions.

Casework Samples

Fifteen known degraded forensic samples were also examined including six samples from the scene of a mass disaster at the Mount Carmel Centre in Waco, Texas [6]. Nine of these samples had been previously examined by HLA-DQA1 and had given either no result or a weak signal. The Waco samples had previously been analysed using the quadruplex STR system [27, 28], however, an exact comparison of success rates could not be made as, due to limited sample availability, a maximum of only two thirds of the original volume was amplified for the SGM. Of the 15 samples 6 gave a full profile, although signals tended to be low and uneven, with a high degree of artefact activity near the amelogenin locus. The results obtained were consistent with those previously observed with DQA1 and the quadruplex system.

Table 4 A summary of the success rates of aged samples tested

Body fluid	Age	No tested	Full profiles
Semen	13 years	1	1
Semen	6 months	12	9*
Saliva	10 years	2	0
Saliva	6 months	16	13
Saliva	15 months	23	12
Blood	1982–1985	24	24
Blood	1986–1990	18	18
Blood	1990–1995	6	5

* Of the three remaining samples, 2 gave partial profiles and Haemotoxylin and Eosin stained slides revealed that a 3rd sample showed no sperm heads suggesting that either a negative area of the stain was tested or that the spermatic fraction was lost during the extraction technique

Ageing

A comparative study of the ageing process on different body fluids stains (Table 4) demonstrated that blood and semen were fairly resistant to the affects of age (Fig. 4). Saliva stains, prepared and examined under identical conditions showed an exaggerated pattern of degradation with preferential amplification of the low molecular weight loci, (particularly HUMTH01) after just 6 months. In all semen and saliva samples, the D8S1179 locus gave the lowest signals and was not present in any of the partial profiles. This locus was also often masked by pull-up[1] under HUMTH01 and high background levels. With these types of samples it would clearly be advantageous to carry out singleplex reactions, in particular to resolve D8S1179. As samples aged the baseline appeared to increase but no significant artefact bands were observed, although a number of small blue artefactual peaks in the Amelogenin and HUMTH01 read regions were observed, none of which could be confused with true HUMTH01 alleles (P. Gill et al., unpublished work).

[1] The dyes in the SGM fluoresce at different wavelengths, however there is some overlap in the emission spectra and to correct for this the analysis software employs a mathematical tool called a matrix. If a wrong or badly made matrix is used or the sample is overamplified then pull-up may be observed as a minor peak directly below a major allele peak, hence a matrix or hardware peak may mask the presence of a true allele peak

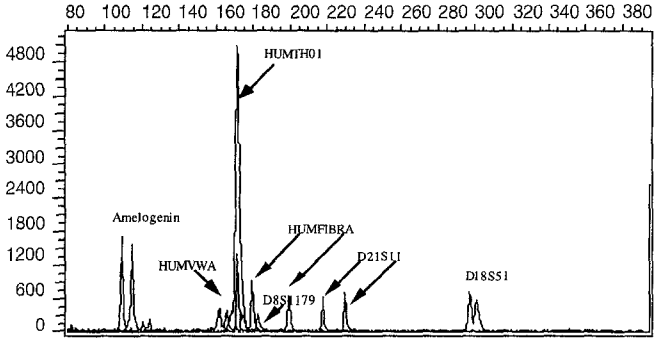

Fig. 4 A full profile was obtained from a semen stain after being stored for 13 years at room temperature (the units on the axis are defined in Fig. 3)

Mixtures

The ABD 373 automated sequencer records signal strength as peak height and peak area, giving a quantitative measure of product yield. Overall it was found that the interpretation of mixtures could be assisted by comparing the peak areas of alleles – although this was difficult when attempting to interpret any mixture with a minor component tenfold less than the major component.

Two different body fluid mixtures were investigated, blood: blood and semen: saliva. In addition, some experiments were carried out where DNA extracted from blood was mixed in known proportions.

Semen: saliva mixtures. A preferential extraction was carried out, and the results obtained reflected the efficiency of the extraction method used to separate the male from the female component, rather than the true mixture ratios (In fact it was rare for any profile originating from the saliva to be observed – the semen component was always easily identified). When the supernatant (epithelial fraction) of a preferential extraction was amplified, mixtures were usually observed, with the semen component consistently low.

Blood: blood and DNA: DNA. There was no difference in the behaviour of mixtures comprised of blood compared to those comprised of DNA. The minor component of a mixture was detected for most loci at 300pg (total DNA- 1ng, ratio 1:2) (Table 5). At 91pg, the lowest level tested (total DNA- 1ng, ratio 1:10), the minor component was only occasionally visible.

Previously [29] it had been reported that it was advantageous to increase the amount of template DNA to identify minor components. However, using the SGM this approach did not usually result in successful typing because of overamplification of the major component. In common with the quadruplex STR system [29] the overlap of stutter bands with an allele was found to increase the peak area of an allele, thus altering the ratio of peak areas at a heterozygote locus.

Both allelic and locus drop out (not related to a specific locus) occurred with the minor component of a mixture at low levels (1:10). Therefore, the possibility of drop out should always be considered when interpreting mixtures. In addition, extensive quantitative work has been carried out to improve the interpretation of mixtures in forensic casework by examination of allele signal ratios, this work will be reported elsewhere.

Table 5 Levels of template DNA amplified for each ratio in the mixture studies. The table shows the quantities used at a total DNA concentration of 1ng, the experiment was also repeated for a total DNA concentration of 5ng

Ratios	1ng
A = 1: 1	500pg : 500pg
B = 1: 2	333pg : 666pg
C = 1: 5	166pg : 833pg
D = 1:10	91pg : 909pg
E = 10: 1	909pg : 91pg
F = 5: 1	833pg : 166pg
G = 2: 1	666pg : 333pg

Discussion

We have previously reported the suitability of a quadruplex STR system for use in routine forensic casework [6, 29]. The SGM system evaluated in this study offers improved discrimination over the quadruplex system (probability of chance association = 1×10^{-8}) with a similar level of sensitivity, and therefore requires the same dedicated conditions to protect against contamination as described previously [29]. DNA from all commonly occurring forensic samples is suitable for analysis with the SGM and profiles were not confused with non-human DNA.

In order to reduce inter PCR variation a bulk multimix was developed where each batch is fully validated before use in order to determine its performance against defined criteria. The quality control procedure includes testing the multimix with matched sets of DNA from known individuals. This has revealed differences in the behaviour of each tissue type, demonstrated in the somatic studies undertaken. To minimise extraction variation, DNA from different tissue types / body fluids from the same set of donors, is extracted in bulk. The profile morphology of these samples is therefore well documented and each batch of multimix can be adjusted to balance the relative locus signals across all body fluids in order to produce a multimix which performs consistently regardless of PCR or multimix batch variations.

In the degradation and ageing studies we established that poor quality samples, especially saliva, have a tendency to show preferential amplification of the lower molecular weight loci, HUMTH01 in particular. However, due to its non-invasive nature saliva was initially proposed for use in the national DNA database. Buccal scrapes, sampling epithelial cells from inside the cheek, by comparison produce a higher yield of DNA reducing many of the problems of degradation. A special multimix, which contains higher levels of D8S1179 primers, was developed for use only on buccal scrapes. The application of the above has seen an increase in the overall success rate of the National DNA Database.

Overall, artefact activity was not found to be problematical with the samples tested. Most artefacts were

observed outside locus-read regions, a few were observed in HUMTH01 and Amelogenein read regions, however, all could be distinguished from true alleles (P. Gill et al., unpublished work). Results detailing studies on actual and mock casework samples (including mixtures) are the subject of a further communication [20] and the effect of variation of amplification parameters and conditions on the efficiency and reproducibility of the SGM has also been evaluated [14].

When analysing poor quality samples the analyst should be familiar with the varied profile morphology that may be expected with forensic samples [6]. With degraded samples or if only limited quantities of DNA of sufficient molecular weight are available the possibility of allelic or locus drop-out must be considered and there may be occasions where it is beneficial to use singleplex tests to assist interpretation. However this was not necessary during this study.

In conclusion the SGM appears to be a reliable, robust and reproducible technique even when tested under extreme conditions.

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