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ORIGINAL ARTICLE

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

(II) Artefacts, casework studies and success rates

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Abstract PCR-based DNA typing of biological evidence is now widely used in forensic analyses due to the obvious advantages of enhanced sensitivity, the ability to distinguish discrete alleles and efficacy with degraded samples. A multiplex short tandem repeat (STR) system has been previously developed which successfully co-amplifies six STR loci HUMTH01, D21S11, D18S51, D8S1179, HUMVWF31/A and HUMFIBRA (FGA) in conjunction with the X-Y homologous gene Amelogenin. This is known as the second generation multiplex system (SGM). Detection of the PCR products is undertaken on ABD 373A or 377 automated sequencers using denaturing polyacrylamide gels coupled with fluorescent-based technology. We have evaluated this system for routine forensic use and demonstrated that the technique is robust and reproducible under conditions consistent with those encountered in a forensic environment. A total of 132 stains from simulated and actual casework were analysed, together with relevant control areas and reference samples. The success rate was high with 76% of stains giving full profiles; we were also able to successfully detect and interpret mixtures. No mistyping was observed. A detailed examination of each of these profiles has assisted in the development of guidelines for casework interpretation. Although artefacts, stutter peaks and undenatured DNA were occasionally observed, these did not interfere with the accuracy of interpretation. In addition 38 samples, previously examined using the quadruplex system, were analysed with the SGM to enable a direct comparison to be made between the systems. The performance of the system with poor quality samples demonstrated its use as a rapid and powerful technique for individual identification.

Key words STRs · Multiplex amplification · Forensic validation · Casework · Individual identification

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Introduction

Given the general quality and quantity of DNA in forensic casework the use of short tandem repeat (STR) analysis of polymorphic loci is the current method of choice for individual identification. The application of polymerase chain reaction (PCR) and automated fluorescence based technology [1] has enabled the development of rapid, sensitive and highly discriminating systems which are proficient in the analysis of highly degraded samples [1] and also have the advantage of definitive allelic identification. In view of recent legislation in the UK initiating the formation of national DNA database, the focus has been on developing multiplex systems which co-amplify multiple loci [1]; whilst these are a compromise of ideal conditions they optimise speed and throughput.

The system described in this communication is a multiplex reaction of three simple and three complex tetranucleotide loci combined with the amelogenin sex test [1]. It is highly discriminating displaying a probability of chance association of 1×10^{-8}. The physical properties and parameters of the system are described by Kimpton et al. [2]. Analysis of old and degraded material, somatic stability, species specificity, and mixtures have been previously reported by Sparkes et al. [1]. This communication will examine the analysis and interpretation of simulated and actual cases. A detailed study of non-allelic artefacts and characteristics of alleles was carried out with a view to designing expert systems to assist the interpretative process (P. Gill et al., unpublished work).

Materials and methods

Preparation of samples

Simulated casework. Blood, saliva, semen and aspermic semen stains, penile and vaginal swabs, from known donors were all prepared, under simulated "casework" conditions on a wide range of substrates. Before the addition of body fluids the garments used were subjected to prolonged wear by the relevant donor and were also exposed to environmental contaminants. Before passing the garments to the donors they had been previously worn, they were soiled and stained and had experienced a high degree of handling. The area of material selected for staining was that most likely to have come into contact with adventitious body fluids, for example crotch areas and armpits. An area adjacent to the stain was also removed for substrate/background testing. Wherever possible intimate samples and stains were generated by natural causes and were not simulated.

Actual casework. Stains and extracts from cases previously tested by DNA single locus probes (SLP) profiling were analysed. The samples were either items with body fluid staining still present or previously extracted DNA.

Comparative study. The 38 samples tested for comparison purposes were actual case stains that were originally submitted to the Metropolitan Police Laboratory for SLP profiling or conventional blood grouping. They were initially examined with the quadruplex system during further validation studies of the system [3].

Preparation of DNA

DNA was prepared from samples using a rapid Chelex extraction [1] and quantified by a dot-blot system which uses a biotinylated probe complementary to a primate-specific alpha satellite DNA sequence (D17Z1) on chromosome 17 [1]. Semen stains, vaginal and penile swabs were preferentially lysed using SDS, proteinase K and DTT as described [4, 5]. The two resulting fractions; both epithelial and spermatozoal, were then incubated with 20% Chelex and quantified as above.

Amplification conditions. The repeat unit and primer sequences for the STR loci have been described previously [1]. Human DNA, between 1 and 3 ng, was amplified in a total reaction volume of 50 µl. This consisted of 1 X PARR buffer (10 mM Tris-HCL pH 8.3, 50 mM KCL, 1.5 MgC_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 (GS500 or GS350) supplied by Applied Biosystems division (ABD). These were heat denatured prior to loading onto a 6% (373A) or 4% (377) polyacrylamide denaturing sequencing gel as described by Kimpton et al. [2]. Gels were electrophoresed for between 5–6 h at constant power (38 or 30 W) on Applied Biosystems automated DNA sequencers models 373A. The running conditions for the 377 sequencer have been previously described [6]. Fragment sizes were determined automatically using Genescan 672 software (ABD) employing the local Southern method [1].

Nomenclature

Nomenclature follows the recommendations of the DNA commission of the International Society of Forensic Haemogenetics with the exception of D21S11. 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: followed by the number of bases of the incomplete repeat: for example HUMTH01 9.3 means this allele has 9 tetrameric repeats plus an additional repeat consisting of 3 bases. The current HUMTH01 designation 9.3 includes the 10 allele as these two alleles differ by only 1 base pair.

Results and discussion

Simulated and actual casework

A total of 247 stains (Table 1), excluding substrate control areas, were extracted at least once. The progression of each sample was closely monitored. Re-extractions, background reactions, and possible inhibitors were all noted and each full profile obtained was closely examined; details of non-allelic peaks, mixtures, stutter bands and n/n + 1 peaks were recorded (see results section).

Simulated cases were designed to be as realistic as possible. Ten cases were prepared, each consisting of various stains commonly encountered in routine forensic casework

Table 1 Breakdown of stains examined and success rates

Sample type	Total no. of stains		Success rates of stains examined				
	Simulated cases	Actual cases	Full	Partial	No profile	Total	Success rates
Blood	47	8	47	3	5	55	85%
Semen	22	12	27	3	4	34	79%
Saliva	22		9	3	10	22	41%
Epithelial	8	2	6	2	2	10	60%
Asp semen	4		4	0	0	4	100%
DNA		5	5	0	0	5	100%
Tissue		2	2	0	0	2	100%
Ref samples	47	68				115	100%
Total			100	11	21	247	
Success rates	72%	89%	76%	8%	16%		

on a wide variety of substrate material (Appendix I). They were processed blind and alleles were designated before comparing the results with reference samples (blood stains or hair roots) provided by the same donors. Interpretation was carried out by scientists experienced in PCR casework and the second generation multiplex STR system (SGM).

All results obtained from simulated casework were as expected. One actual case consisted of a complex mixture from three individuals, all of the alleles present in the accused's profile were found in the stain; however it was apparent that some alleles were masked by the presence of a second, stronger component not previously detected. Interestingly the component proportions of the mixture varied according to the area of the stain tested. All other actual cases (Appendix II) gave results entirely consistent with those previously obtained with single locus probe (SLP) DNA profiling.

The success rate for actual casework, excluding reference samples was 89% (full profiles) whereas the success rate for simulated casework was 72% (Table 1). The trend observed in the performance of individual body fluids was in common with validation studies previously undertaken [1] i.e. saliva stains gave the lowest overall success rate.

Comparative study

Examination of the results obtained from the comparative study (Table 2) revealed that there is very little difference between the sensitivity of the two systems. When considering a direct comparison of performance, it is worth considering the time delay between analysis. This will increase the chance of sample failure with the SGM, as storage time and repeated thawing both assist in DNA degradation.

Table 2 Direct comparison of success rates obtained between the quadruplex system, currently in routine forensic use in the UK and the second generation multiplex system being validated. Samples previously examined during the validation of the quadruplex system by Andersen et al. (1995) were analysed using the SGM

1) Quadruplex results			
Body fluid	Full	Partial	No result
Blood	14	4	0
Semen	13	0	1
Epithelial	4	1	0
Saliva	1	0	0
Total	32	5	1
Success rate	84%	13%	3%
2) SGM results			
Body fluid	Full	Partial	No result
Blood	14	3	1
Semen	12	1	1
Epithelial	5	0	0
Saliva	1	0	0
Total	32	4	2
Success rate	84%	11%	5%

Substrate 'controls'

Analysis of substrate controls adjacent to a crime stain has been suggested for STRs as a control to test for the possibility of background 'contamination' [1] where contamination in this context refers to transfer of DNA from an individual other than the donor of the crime stain itself. The control area is taken from a region close to the stain.

An obvious source of transfer would be from the victim, particularly on clothing subject to the greatest degree of bodily contact e.g. cuffs, pockets, crotch. Comey and Budowle [7] and Lygo et al. [8] carried out experiments to examine the effect of deliberate handling or coughing onto exhibits, concluding that passive transfer of DNA is difficult to achieve and unlikely to confuse the profile of the main crime stain (under standard conditions used in forensic laboratories). In our simulated case study 69 control areas adjacent to the stain were analysed (Appendix III). Negative results were obtained from 58 of the samples, 2 gave full profiles and 9 gave partial profiles with HUMTH01 present in every case. In 6 samples the profile obtained was the same as the wearer and 6 cases revealed DNA present from an unknown source – possibly indicative of more than one wearer of garment. Despite the presence of background DNA in the controls, it was always at a low level and never interfered with interpretation of the stain itself. Two controls, both taken from a pair of knickers, gave profiles that were the same as the stain, but at much lower level. This was consistent with diffusion of the stain into the control area.

The significance of a 'substrate' result in relation to interpretation of the main crime stain has been further explored and will be the subject of a further communication (P. Gill, in press, For Sci Int, 1996).

Interpretation of results

Mixtures

Out of 100 full profiles examined 24 were mixtures of varying signal strength. In the majority of cases interpretation of the mixtures was possible and reference samples confirmed initial designations (Fig. 1).

The simulated casework included 41 stains which were generated using a mixture of body fluids, the ratio of mixture components from stains not generated by natural causes were estimated to be in roughly equal proportions (Table 3). An extraction technique used to preferentially separate the male and female component was employed with 14 samples, this method results in two DNA supernatants which can each be analysed; a preferential fraction containing spermatic DNA and an epithelial fraction potentially containing DNA from both male and female epithelial cells. The preferential fraction from these 14 samples was analysed and as expected, in every case the male

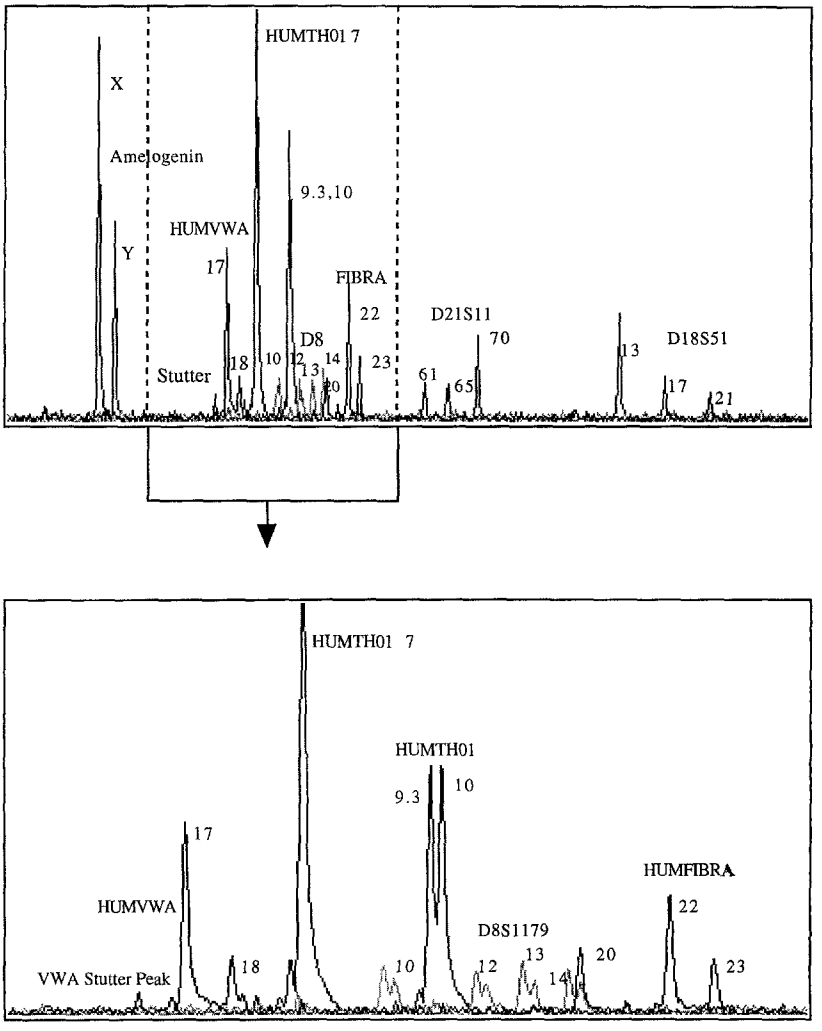

Fig. 1 A profile obtained from a mixed sample consisting of blood (male) and saliva (female) in approximately equal proportions. A good indication of the proportion of allelic components is obtained by examination of the peak areas. For example the homozygous HUMTH01 7 peak is twice as great as the heterozygous HUMTH01 9.3 and 10 peaks. Similarly, a triple dose of HUMVWA allele 17 is indicated as the peak area is 3X the size of allele 18. If it is not possible to distinguish between major and minor components then all the possible genotypes are considered following the method of Evett et al. (11)

Designations of mixed profile and reference samples

	Amelogenin	HUMTH01	D21S11	D18S51	D8S1179	HUMVWA	HUMFIBRA
MALE - Blood	X,Y	7	61,65	13,21	10,14	17,17	20,22
FEMALE - Saliva	X,X	9.3,10	70,70	13,17	12,13	17,18	22,23
STAIN	X,Y	7,9.3,10	61,65,70	13,17,21	10,12,13,14	17,18	20,22,23

Table 3 Breakdown of mixed body fluid stains. All stains were prepared as detailed in the materials and methods.

Body fluid mixture	No of mixed stains	Mixed profile obtained	Comments
Semen/vag secretions – preferential*	14	2	2 mixtures were weak female component
Semen/vag secretions – epithelial*	5	3	
Aspermic sem./vag secretions	7	0	No profiles from aspermic sem. detected
Saliva/Blood	4	3	No distinct major component detected
Semen/Blood	8	4	No distinct major component detected
Blood/blood	2	1	
Saliva/epithelial cells	1	1	
Total	41	14	

*Preferential indicates that DNA from the male seminal component was amplified.
*Epithelial indicates that DNA from the epithelial fraction obtained using a preferential extraction was amplified, this may also contain epithelial cells from the male component.

component was dominant with only two samples showing a weak female contribution to the profile. We were unable to differentially extract the remaining 27 samples however, in more than 50% of the samples, there was a marked tendency for only one body fluid profile to be apparent.

Overamplification

A sample is considered overamplified if allele product heights are greater than 4000 units when run on an ABD 373A sequencer. Accurate quantitation should prevent the

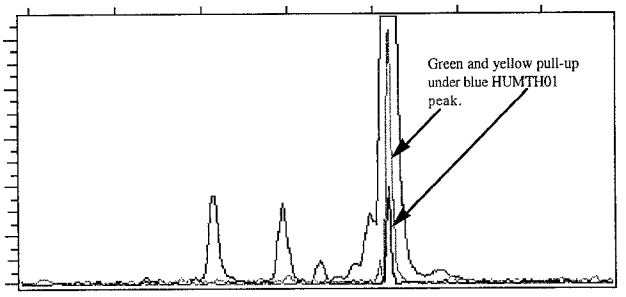

Fig. 2 Pull-up under HUMTH01 in a degraded sample where HUMTH01 has overamplified. The dyes in the SGM fluoresce at different wavelengths, however there is some overlap in the emission spectra, to correct for this the analysis software employs a mathematical tool called a matrix. If a wrong or badly 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. The size of the products in bases is shown along the *x* axis and the *y* axis shows the peak height in arbitrary units

amplification of excessive DNA, however overamplification was detected in approximately 5% of samples, predominantly in reference blood. Overamplification is recognised by an excessive number of artefact peaks and stutter bands together with a high incidence of background activity and "pull-up" – defined as a minor peak directly below a major allele peak (Fig. 2). In this study, dilution of the PCR product was sufficient to produce an accurate profile and reamplification was not necessary, however reamplification of a lesser quantity of DNA may be required if excessive artefacts are present.

Non-allelic peaks

Stutter bands

Slippage of the Taq polymerase enzyme during primer extension results in artefactual 'stutter' peaks one repeat unit smaller than the true allele product. Most stutter bands appear approximately 4 base pairs (bp) lower than the main allele peak. Further analysis of the stutter characteristics of these samples will be reported elsewhere (P. Gill et al., unpublished work). Occasionally 2 bp stutters were observed, in association with a 4 bp stutter, at the D18S51 and D21S11 locus (out of 219 samples 3 at D18S51 and 1 at D21S11), they were consistently lower than the 4 bp stutters.

Undenatured DNA

Incomplete denaturing conditions may result in atypical peak morphology or the formation of extra small undenatured DNA peaks associated with the main allele peak (Table 4). Peak morphology of undenatured DNA was less well-defined than that of denatured products (Fig. 3). Several samples also had small peaks 4 base pairs greater than the true allele peak, these were initially thought to be "overstutters", however subsequent analysis revealed that

Table 4 Sizing windows of undenatured DNA. Although the position of an undenatured DNA peak could not be definitively predicted, the approximate position of the peak in relation to the associated allele was found to be reproducible for each locus within a set size window

Locus	Size window reduction in base pairs	Based on no. of results
Amelogenin	X = 6–12	23
	Y = 7–15	23
HUMTH01	38	9
D21S11	17–23	74
	27–30	
D18S51	24–29	69
	32–36	
	39–43	
D8S1179	8–11	70
	16–19	
HUMVWF31/A	10–14	74
	17–20	
HUMFIBRA	12–16	72
	20–22	

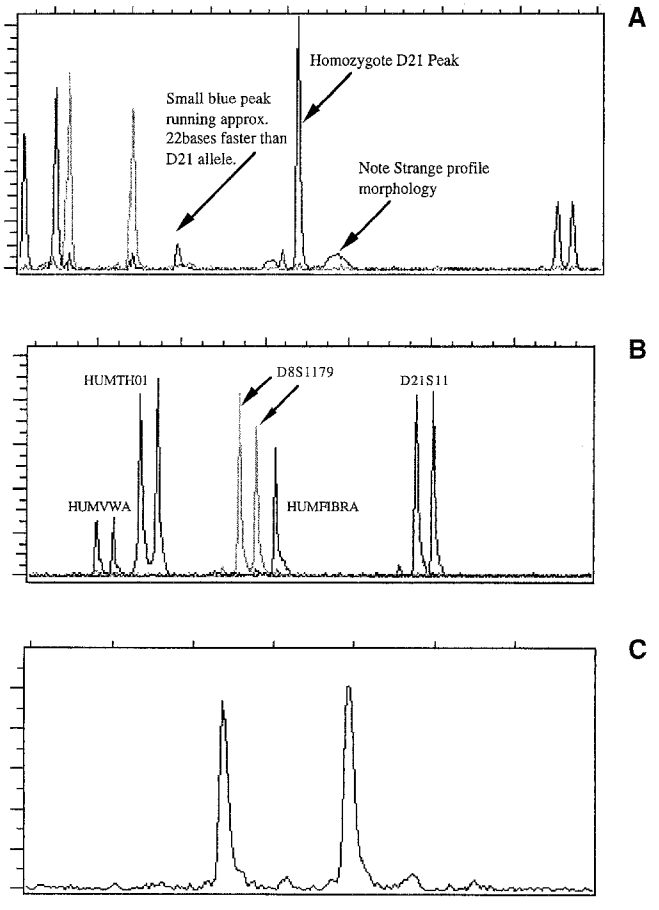

Fig. 3 A–C The different effects of undenatured DNA. **A** The homozygous D21S11 allele shown is associated with a small blue undenatured DNA peak running approximately 22 bases faster, the locus also demonstrates the atypical peak morphology seen with undenatured DNA. **B** shows the decreased back slope gradient often observed when DNA is not completely denatured, it is visible at all loci shown but most prominent at HUMFIBRA. **C** demonstrates undenatured DNA giving the appearance of "over stutters" at the HUMFIBRA locus. (The units on the axis are defined in Fig. 2)

these artefactual peaks were also caused by incompletely denatured DNA. Re-running the PCR product, when properly denatured, was sufficient to eliminate all of the artefacts described. Early identification of these characteristic artefacts may be confirmed by an overview of the gel, undenatured DNA was often observed as a trend across an entire gel, and was generally more apparent at a homozygous locus, particularly HUMFIBRA.

Results have demonstrated that reannealing of DNA is less likely to occur with the ABD 377 sequencer. This is probably due to the increased running temperature employed by these machines which assists in preventing the DNA from reannealing [6].

N and N+1 peaks

The true length allele signal is known as the 'n' peak. The addition of an extra base by the Taq polymerase enzyme at the end of the elongation step can produce a PCR product 1 base larger than the true allele length and this is termed the 'n+1' peak [9, 10]. The amplification parameters of the SGM are designed to encourage the formation of 'n+1' peaks in the form of a double or split peak. The relative intensity of the 'n' signal is normally lower than the 'n+1', however this may not always be the case especially at the D8S1179 locus (Fig. 4).

During the course of this validation study 'n' peaks were observed at each locus, however the frequency of occurrence (Table 5) and the ratio of true length (n) to true length-plus-1-base (n + 1) was largely locus dependant (Fig. 4).

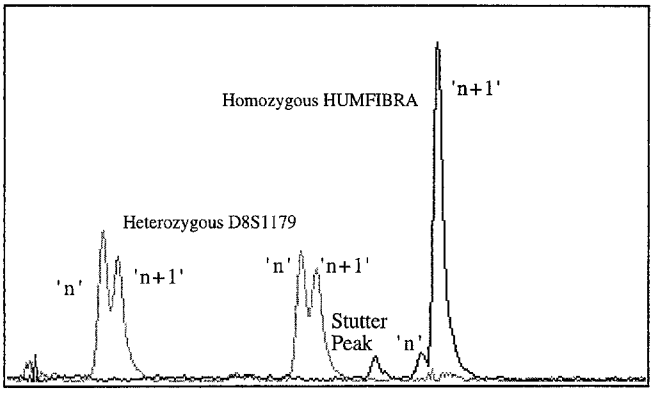

Fig. 4 Different profile morphology of n+1 peaks. The locus D8S1179 displayed the highest percentage of double peaks and the ratio of n:n+1 was approximately 1:1. HUMFIBRA also displayed a high number of 'n' peaks however the approximate ratio of n:n+1 was substantially lower at 1:10

Accurate interpretation was not affected by the presence of 'n' peaks, in fact the distinct double peak morphology displayed by the D8S1179 locus often confirmed the presence of an allele rather than an artefactual peak. This was particularly applicable in cases of overamplification of HUMTH01, where green pull-up under a large blue peak may fall into the D8S1179 read region (Fig. 2).

Non-specific artefacts

Any remaining non-allelic peaks that could not be classified as a stutter band, undenatured DNA, an 'n' peak or pull-up may be defined as a non-specific artefact. During the course of this study artefact bands were often observed, however they did not affect interpretation of results and the majority fell outside of a locus read region. A detailed examination of these bands and their effect on profile interpretation will be described elsewhere (Gill et al., unpublished work).

Conclusion

This study forms part of our laboratories detailed validation process of the SGM system and has examined the performance of the technique on both simulated and actual forensic casework samples. Where possible between 1 and 3 ng of DNA was amplified for all samples, however on several occasions the concentrations of DNA obtained from the alpha satellite quantitation were such that it was necessary to amplify levels of DNA below 1 ng. On these occasions full and partial profiles were obtained with allele signals close to threshold. When interpreting profiles with allele signals close to threshold the relevance of the profile should be carefully considered. The substrate control experiment demonstrated that DNA from an 'unknown' source (i.e. neither the wearer nor the suspect) was detected in six stains all of which had given quantitation results of less than 250 pg.

The simulated study was designed to cover the worst case scenarios; materials known to inhibit, such as denim and suede fabric, soil and rust, were selected as substrate materials (Appendix I), excessive handling and wearing of the garments also ensured that every effort was made to expose the substrate material to possible human DNA contamination. In addition, many of the stains consisted of a mixture of body fluids. In all cases the SGM was shown to be reliable and robust with a total of 72% of samples yielding full profiles and 9% yielding partials. In-

Table 5 Frequency of occurrence of 'n' peaks. The total number of profiles examined was 219

Locus	Amelogenin	HUMTH01	D21S11	D18S51	D8S1179	HUMVWF31/A	HUMFIBRA
Total no of alleles with 'n' peaks	17	30	4	1	110	3	61
Percentage of 'n' peaks	8%	14%	2%	0.5%	50%	1.5%	28%

terestingly, the level of background activity observed in the substrate controls and the number of mixed profiles obtained was lower than anticipated.

The success rate of the actual case samples was substantially higher: the actual casework experiment showed that 89% gave full profiles and 7% yielded partials; the comparative study also showed that 84% gave full profiles and 11% yielded partials. As with the simulated investigation the technique was shown to be reproducible and generally profiles were of a high quality despite the limited amount of sample material available.

This paper, in conjuction with previous publications [1] have offered recommendations that assist in the interpretation of all profiles, including mixtures. The overall conclusion from these detailed studies is that the SGM is a highly discriminating and reliable individual identification tool suitable for both routine forensic applications and intelligence database construction.

Appendix I

Simulated casework summary

Item	No	Stain	Substrate
QMC1/94			
Envelope	2	Saliva	Brown HMSO 7 cream private
Stamp	3	Saliva	2 1st class & 1 2nd
Cigarette Butt	9	Saliva	
Ref Blood	8		
QMC2/94			
Knickers	2	Semen	Black 45% cotton/55% viscose
Cardigan	1	Semen	Black acrylic
Int Vag Swab	1	Semen	
Penile swab	2	Epithelial	
Wood.	3	Blood	
Trainers	6	Blood	
Shirt	3	Sal & bld mix	65% Polyester/35% cotton
Ref Blood	4		
QMC3/94			
Balaclava	1	Saliva	Black acrylic
Stocking mask	1	Saliva	Nylon
Leather jacket	2	Blood	Brown leather
Jeans	1	Blood	Light blue denim
Shoe instep	2	Blood	Brown suede
Ref blood	4	Ref blood	
QMC4/94			
Breast swab	1	Saliva	
Condom i/s	1	Semen	Mates
Condom o/s	1	Epithelial	
Tights	3	Blood	Brown Nylon
Jeans	1	Blood	Dk blue denim
Leaf	3	Blood	Lng lily/round green/variegated
Tissue.1	1	Blood	Toilet paper
Tissue.2	1	Semen	Toilet paper
Track suit	1	Blood	Black 65% cotton/35% polyester
Ref Blood	4		
QMC5/94			
Sweatshirt	1	Blood	Orange cotton
Metal bar	1	Blood	Rusty metal
Vaginal swab	2	Semen	inc. lubricant
Knickers	1	Semen	cotton
Ref Blood	4		
QMC6/94			
Leggings.1	1	Semen	Black lycra
Leggings.2	1	Saliva	Black lycra
Leggings.3	1	Blood	Black lycra
QMC6/94			
Trainers	2	Semen	
Trainers	2	Blood	
Scarf	1	Semen	Blue wool
Wood	1	Semen	Wood
Int vag swab	1	Semen	& Vaseline
Jumper	1	Blood	Black acrylic
Ref blood	4		
QMC7/94			
Knickers	1	Aspermic Sem.	Nylon
Int vag swab	1	Aspermic Sem.	
Belt	2	Blood	Leather
Jeans	1	Saliva	Dark blue denim
Jumper	1	Blood	Wool/nylon
Cords	1	Blood	98% cotton/2% lycra
Wellington	1	Blood	Rubber
Ref blood	4		
Ref hair	1		
QMC8/94			
Condom i/s	2	Semen	Coloured & ribbed Durex
Condom o/s	2	Epithelial	Coloured & ribbed Durex
Knickers	1	Semen	Silk
Penile swab	1	Epithelial	
Breeze Block	2	Blood	Stain 2 includes ninhydrin
Sweatshirt	1	Blood	65% polyester/35% cotton
Jacket	1	Saliva	Faded denim
Ref Blood	4		
QMC9/94			
Knickers.	2	Semen	100% polyester
Int Vag Swab	2	Semen	With KY jelly
Poloneck	1	Blood	100% cotton
Knife	1	Blood	Metal blade
Jumper	1	Sal/bld mix	100% wool
Ref blood	4		
QMC10/94			
Knickers	1	Aspermic Sem.	Cotton mix
Int vag swab	1	Aspermic Sem.	
Sweatshirt	1	Blood	90% Red polyester
Trousers	1	Blood	Pocket lining
Football shirt	1	Blood	?-Stretchy material
Penile swab	2	Epithelial	
Chair leg	3	Blood	Varnished wood
Ref blood	6		

Appendix II

Summary of actual cases

Case No	Item	No	Stain	Case No	Item	No	Stain
QBC2	Tissue	1	Semen	QBC16	Facecloth	2	
	Tissue	1	Epith.		Cardigan	1	
	Ref bloods	4			Ref bloods	2	
QBC3	Knickers	1	Semen	QBC17	Pants	1	DNA
	Knickers	1	Epith.		Ref bloods	4	
QBC5	IVS.	1	DNA	QBC18	Floorboard	1	DNA
	Ref bloods	4			Ref bloods	2	
QBC7	Vulval swab	1	DNA	QBC21	Flesh	1	Tissue
	Ref bloods	4			Ref hair	2	
QBC8	IVS	1		QBC22	Flannel	2	Semen
	Ref bloods	4			Ref bloods	4	
QBC11	Jeans	1	Blood	QBC23	Jersey	1	Blood
	Shirt	1	Blood		Ref bloods	4	
	Jumper	1	Blood	QBC24	Nightie	1	Semen
	Ref bloods	2			Ref bloods	4	
QBC12	Foetal tissue	1	Tissue	QBC25	Jeans	1	
	Ref bloods	4			Ref bloods	4	
QBC13	LVS	1	Semen	QBC27	Mattress	1	Semen
	HVS	1	Semen		Sanitary Towel	1	Semen
	Ref bloods	4			Ref bloods	4	
QBC14	Knickers	1		QBC28	Skirt	1	
	Ref bloods	4			Ref bloods	4	
QBC15	Knickers	1					
	Ref bloods	4					

Abbreviations. IVS Internal vaginal swab
EVS External vaginal swab
HVS High vaginal swab

Appendix III

Summary of 'substrate' controls

Case No	Item	No	Stain	Result							Same as or different to adjacent stained area.
				Am	TH01	D21	D18	D8	VWA	FGA	
QMC2	Knickers	2	Semen	Negative							
	Cardigan	1	Semen	Negative							
	Wood	3	Blood	Negative							
	Rt. trainer.1	1	Blood		9.3	70	13, 17	13		22	Different-wearer
	Rt. trainer.2	1	Blood	Negative							
	Lt. trainer.1	1	Blood	Negative							
	Lt. trainer.2	1	Blood	Negative							
	Lt. trainer.3	1	Blood	X(X,Y)	6, 9, 9.3	59, 63, 70	13, 17, 19		17, 18, 19	18, 21, 22, 23	Different-wearer & unknown
	Lt. trainer.4	1	Blood	Negative							
	Shirt. 1	1	Sal & Bld	Negative							
	Shirt. 2	1	Sal & Bld	X,X	9.3	65	12, 13, 14		19		Different–unknown
	Shirt. 3	1	Sal & Bld	Negative							

Appendix III (continued)

Case No	Item	No	Stain	Result							Same as or different to adjacent stained area.
				Am	TH01	D21	D18	D8	VWA	FGA	
QMC3	Balaclava	1	Saliva	Negative							
	Stocking mask	1	Saliva	Negative							
	Leather jkt	2	Blood	Negative							
	Jeans	1	Blood	Negative							
	Shoe instep	2	Blood	Negative							
QMC4	Tights.1	1	Blood	XX	9.3						Wearer/stain -- NR
	Tights.2	1	Blood	Negative							
	Tights.3	1	Blood	XX	9.3	63, 65, 67		13		18, 19, 22.2	Different-wearer & unknown
	Jeans	1	Blood	Negative							
	Leaf	3	Blood	Negative							
	Tissue.1	1	Blood		6	65					Different
	Tissue.2	1	Semen	Negative							
	Track suit	1	Blood		6, 9.3						Different
QMC5	Sweatshirt	1	Blood	Negative							
	Metal bar	1	Blood	Negative							
	Knickers	1	Semen	Negative							
QMC6	Leggings.1	1	Semen	Negative							
	Leggings.2	1	Saliva	X,X	6, 9	59, 63	13, 19	13	18, 19	18, 21	Different-wearer
	Leggings.3	1	Blood		7, 9	59, 63					Different
	Trainers	2	Semen	Negative							
	Trainers	2	Blood	Negative							
	Scarf	1	Semen	Negative							
	Wood	1	Semen	Negative							
	Jumper	1	Blood	Negative							
QMC7	Knickers	1	Asp Sem	X,X	7, 7	65, 69	14, 16	10, 13		22, 23	Same as stain-wearer
	Belt.	2	Blood	Negative							
	Jeans	1	Saliva	Negative							
	Jumper	1	Blood	Negative							
	Cords	1	Blood	Negative							
	Well. boot	1	Blood	Negative							
QMC8	Knickers	1	Semen	Negative							
	Breeze block	2	Blood	Negative							
	Sweatshirt	1	Blood	Negative							
	Jacket	1	Saliva	Negative							
QMC9	Knickers.1	1	Semen	X, Y	6, 7	61, 65	12, 19	10, 14	16, 19	23, 24	Same as stain
	Knickers.2	1	Semen	Negative							
	Poloneck	1	Blood	Negative							
	Knife	1	Blood	Negative							
	Jumper	1	Sal/Bld	Negative							
QMC10	Knickers	1	Asp Sem	Negative							
	Sweatshirt	1	Blood	Negative							
	Trousers	1	Blood	Negative							
	Football shirt	1	Blood	Negative							
	Chair leg	3	Blood	Negative							

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