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Validation of the AMP*Fl*STR® SGM Plus™ system for use in forensic casework

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Abstract

The AMP*Fl*STR® SGM Plus™ system is a commercially available STR multiplex produced by Applied Biosystems, a division of Perkin Elmer, Foster City, California, USA that supersedes SGM. The multiplex contains the six SGM loci, amelogenin and four additional loci. These additional loci are D3S1358, D19S433, D16S539 and D2S1338. Consequently, the match probability is significantly improved (conservatively quoted as 1 in 10^9 for reporting a full profile match). The system was subjected to validation. For example, ageing and degradation studies demonstrated semen stains to be the most stable evidence type, whereas buccal scrapes were the least stable. An apparent rise in the sensitivity increases the chance of obtaining successful results from the more difficult samples submitted for analysis. Two of the new loci (D3S1358 and D19S433) are low molecular weight (between 100 and 150 base pairs); this improved the success rates of the degraded samples where high molecular weight loci may drop out. Of 26 non-primates tested, four gave results that appeared as single peaks and were unlikely to cause interpretation problems. None of the 19 micro-organisms tested gave discernible results. Extensive casework and simulated casework studies demonstrated that SGM and SGM plus results were comparable. There was one example of a null allele (primer binding site mutation) recorded at the HUMFIBRA locus (in both systems). However, a concordance study of 1000 samples using both SGM and SGM plus did not demonstrate any differences in typing.

Keywords: DNA; Short tandem repeats (STRs); Multiplex

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1. Introduction

The AMP*Fl*STR® SGM Plus™ (Perkin Elmer Biosystems, Foster City, CA, USA) is a ten locus multiplex that comprises the six second generation multiplex SGM loci and the amelogenin sex test [12,13] alongside four additional loci – D3S1358 [9], D16S539 [1], D2S1338 [19] and D19S433 [8]. These are compound tetranucleotide loci [15]; the match probability is conservatively given as 1 in 10^9 when reporting a full profile match [3]. D3S1358 and D19S433 are low molecular weight and are less susceptible to locus dropout when degraded samples are analysed. In addition, the SGM loci and D3S1358 are standards that have been recommended by the European Network of Forensic Science Institutes (ENFSI) to enable pan-European comparisons of STR profiles [5]. The primer pairs and fluorescent dyes for the SGM and comparable SGM Plus loci differ, although the Amelogenin primers are the same. The system is validated for use in forensic casework, by the manufacturer, to the guidelines recommended in the Technical working group on DNA Analysis methods (TWGDAM) [11,14]. We describe validation studies performed to test the robustness and reproducibility of the AMP*Fl*STR® SGM Plus™ multiplex on samples routinely found in casework, including old and degraded samples.

2. Materials and methods

2.1. Preparation of samples

Where possible, stains previously used for SGM validation [12,13] were analysed, along with new blood, saliva and semen stains, on Guthrie cards, that had been prepared from fresh liquid samples. For the somatic studies, matched sets of blood, saliva, hair, buccal scrapes and vaginal swabs or semen were prepared. Samples for a simulated casework study were prepared as described by Sparkes et al. [13].

2.2. Species specificity

Samples were the same as described by Sparkes et al. [12] and included higher primates, domestic animals, farm animals and micro-organisms. The quantification values ranged from 1 to 100 ng.

2.3. Ageing and degradation studies

Four groups of samples stored at normal room temperature and humidity were investigated. These were:

1. ten blood stains prepared at different times since 1982 (oldest stain: 17-years-old, newest stain=9-years-old).

2. ten semen stains ranging from 5 to 11-years-old.
3. six saliva stains ranging from 5 to 17-years-old.
4. four buccal samples from 1995.

In addition, blood, saliva and semen stains were prepared and placed in a plastic box at 100% humidity in a 37°C incubator. The stains were sampled at intervals between 1 and 5 days up to a maximum of 60 days.

2.4. Casework studies

The extracts for the simulated casework study were as described by Sparkes et al. [13]. Where necessary, new extracts were prepared.

2.5. Preparation of DNA

The rapid chelex extraction method described by Walsh et al. [16] was used, except that prior to use, 6 g of chelex was washed with 30 ml of HPLC grade Sigma water (cat no. 27 073-3, Sigma-Aldrich, Dorset, UK). This stock solution of 20% chelex could be diluted to a 5% concentration where needed. Quantification was performed by either the reverse dot blot method [17] or by picogreen [6].

2.6. Amplification conditions

An aliquot of 1 ng of template DNA was amplified in a total reaction volume of 50 μl. The AMP*Fl*STR® SGM Plus™ kit contained reaction mix, primer mix (for components see Perkin Elmer user manual [11]), Amplitaq Gold®at 5 U/μl and AMP*Fl*STR® Control DNA (Identifier: 007 –0.10 ng/μl of male DNA, heterozygous at all loci in 0.05% sodium azide and buffer). Samples were amplified in 0.2-ml bubble top tubes (supplied by PE Biosystems cat no: N801-0612) without mineral oil on a 9600 thermal cycler (Perkin Elmer) using the following conditions (recommended by the manufacturer): 95°C for 11 min, 28 cycles of 94°C for 1 min, 59°C for 1 min and 72°C for 1 min, 60°C extension for 45 min and 25°C for up to 18 h.

2.7. Detection

A 1-μl aliquot of amplified DNA product was combined with 1 μl of dextran blue-formamide mixed with internal size standard labelled with the dye ROX (Rox XL500-PE Biosystems, Foster City, CA, USA) in a ratio of 8:1. This was heat denatured on a 9600 thermocycler and snap-cooled on ice. An allelic ladder, containing amplified alleles for all loci, provided with the AMP*Fl*STR® SGM Plus™ kit was electrophoresed in two lanes (in one odd lanes and one even) of a gel made of 5% Longranger acrylamide–6 M urea mixture (FMC Bioproducts, Rockland, ME, USA). Electrophoresis was carried out for 2.5 h using an automated DNA sequencer (model 377 PE

Biosystems, Foster City, CA, USA). The raw data was analysed using Genescan® 2.1.1 and Genotyper® 1.1.1 software (PE Biosystems, Foster City, CA, USA).

2.8. Nomenclature

Using the Genotyper software version 1.1, all loci were designated according the number of repeat units present as recommended by the DNA Commission of the International Society of Forensic Haemogenetics [2].

3. Results and discussion

3.1. Extraction

It was found that (possible) PCR inhibitors in chelex affected *Taq* gold polymerase used with SGM Plus™, thereby reducing sensitivity and the quality of the profile obtained (Sean Walsh; personal communication). AmpliTaq used with SGM appeared not to be affected to the same degree. Inhibitors were removed by washing the chelex in distilled water (Fig. 1).

The validation detailed in this paper was carried out to determine the robustness and reliability of the AMP*Fl*STR® SGM Plus™ under different casework conditions. For the majority of investigations, direct comparisons were made with the SGM system. Results from the initial optimisation experiments performed at the Forensic Science Service gave a good indication that the AMP*Fl*STR® SGM Plus™ was more sensitive than SGM and should therefore provide results with degraded or small DNA samples where no result or a partial result was obtained with SGM.

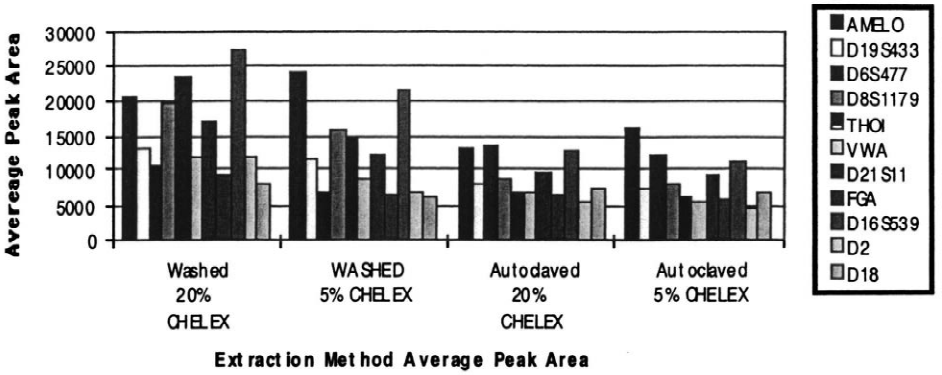

Fig. 1. Peak areas at each locus were averaged across ten samples to show the differences between the different extraction methods.

3.2. Species specificity

The results of the primates tested showed that amplified product was detected with bands appearing in the read region (90–450 base pairs). The positive results are shown in Table 1.

Of the non-primates tested with AMP*Fl*STR® SGM Plus™ only four species showed any peaks within the 90–450 base pair read region. These were:

1. Cat Green peak at 146 base pairs (D8S1179 12.1). Blue peak at 147 base pairs (D3SS1358 19.3)
2. Dog Green peak at 102 base pairs (Amelogenin region)
3. Lamb Green peak at 102 base pairs (Amelogenin region)
4. Rabbit Green peak at 106 base pairs (Amelogenin X)

The non-primate species which gave no results when tested were: Herring; Sea trout; Frog; Toad; Chicken; Duck; Goose; Hen; Pheasant; Pigeon; Turkey; Badger; Calf; Elephant; Fox; Guinea pig; Horse; Mouse; Pig; Rat; Red deer; Sika deer;

The micro-organisms tested were: *Bacillus anthracis* 245; *Bacillus cereus*; *Bacillus mycoides*; *Bacillus stearothermophilus*; *Bacillus thuringiensis*; *Clostridium acetobutylicum*; *Clostridium barati* F; *Clostridium botulinium* A; *Clostridium botulinium* B; *Clostridium botulinium* G; *Clostridium butyricum*; *Clostridium difficile*; *Clostridium perfringens*; *Clostridium sporagenes*; *Clostridium therniocellum*; *Escherichia coli* B; *Pseudomonas fluorescan*; *Staphylococcus aureus*; *Thermus aquaticus* XT1.

None of these micro-organisms gave any discernible peaks. The 102 base pair peak that was seen in SGM was probably caused by the amelogenin primers. The 102 base pair peak appeared with SGM Plus™ in some domestic animals as a green peak instead of blue because of the dye change at amelogenin.

3.3. Ageing and degradation studies

3.3.1. Blood/saliva stains

Saliva stains showed the greatest susceptibility to degradation out of the sample types investigated. The most severely affected were aged buccal scrapes. All showed either partial or complete drop out of the high molecular weight loci (Figs. 2 and 3)

3.3.2. Semen stains

Old semen stains were much more stable compared to blood and saliva. When degradation was observed, the profile showed imbalance. For unknown reasons HUMVWF31/A was characteristically preferentially amplified.

Similar effects were observed when samples were stored in a plastic box at 100% humidity. Blood stains showed signs of degradation after 25 days, saliva after 28 days and semen after 42 days.

Table 1
A summary of the peaks observed when primate DNA was amplified with the AMP*Fl*STR® SGM Plus™ system[a]

Species	Amelogenin	D8S1179	D21S11	D18S51	HUMVWF31/A	HUMFIBRA	D3S1358	HUMTHO1	D2S1338	D16S539	D19S433	Out of Locus range
Great apes												
Chimpanzee 'Bobby'	X(106.53)	10(135.52)	181.33		12.0(161.13)	17.3(219.34)	14(122.34)	6(173.73)	21(316.38)	9.3(252.69)		
(*Pan troglodytes*)	Y(112.14)	12(144.20)	185.45			23.3(244.20)		7(177.77)	22(320.15)			
Gorilla	X(106.53)	124.32			17.3(183.99)	27.3(260.31)	16(130.47)	4(165.85)	22(320.41)	11.3(260.65)		
(*Gorilla gorilla*)						32.1(277.98)	17(134.68)	7(177.7)	24(328.42)	12.3(264.54)		
Orangutan	105.45	116.26				24.3(246.72)	18(139)			11.2(259.68)		
(*Pongo pygmaeus*)												
Old world monkeys												
Celebes black Ape	X(106.93)					26.2(255.38)						
(*Cynopithecus niger*)												
New world monkeys												
Owl monkey	X(106.35)											
(*Aotes trivirgatus*)												
Prosimians												
Hanuman langur	X(106.51)	109.87										
(*Presbytis entellus*)												
Gibbon	107.61	11(139.12)			11(156.7)							
(*Hylobates lar*)		12(143.64)			13(164.44)							

[a] The sizes are based on those observed when electrophoresed on a 377 sequencer and are hence only approximate guidelines. Where possible, the peaks have been attributed to an allelic window or a locus range if outside of the allelic windows.

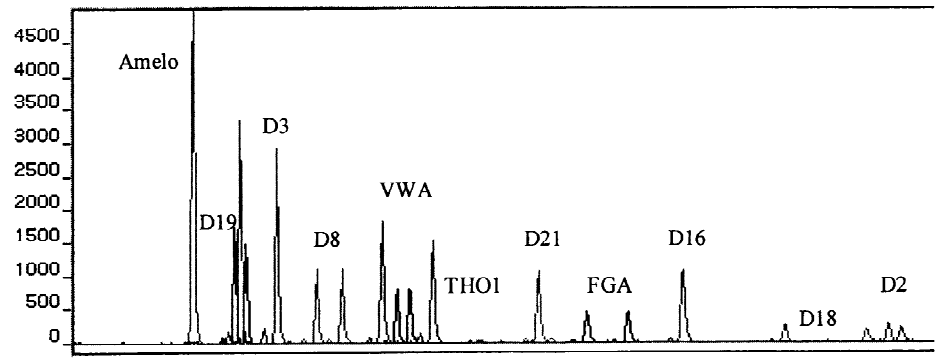

Fig. 2. Seventeen-year-old blood stain stored at ambient room temperature and humidity showing degradation pattern of decreasing peak area with increasing molecular weight. The typing results were as expected and no allele or locus dropout was observed.

3.4. Somatic studies

Blood, buccal, hair, saliva and semen or vaginal swab (semen free) samples from seven known individuals, (four male donors and three female donors) were amplified and analysed to test somatic reproducibility and stability. The results showed no apparent differences in any of the samples and the profiles were generally well balanced both within and between loci.

No artefacts were detected in any of the sample types and the baseline showed little noise. Any stutters that occurred in the profiles were below 10% of the associated allele and were not prevalent in any sample type.

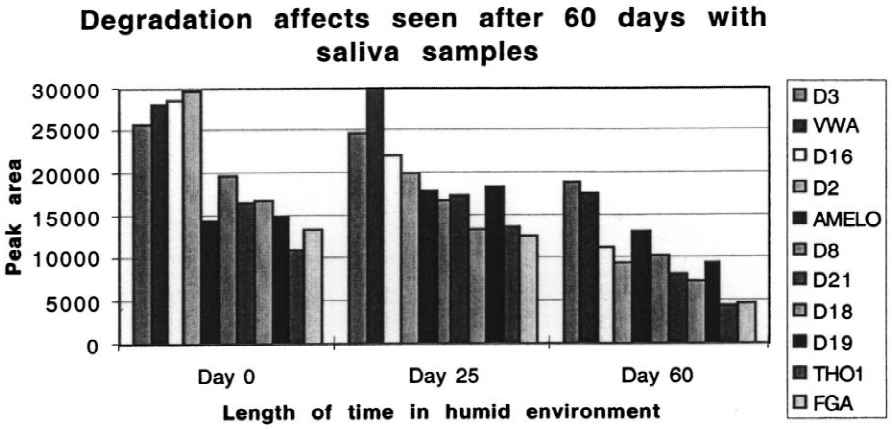

Fig. 3. The peak areas from all the degraded saliva samples were averaged for each locus. Looking at each dye label, a clear degradation pattern can be observed at day 60 across the loci from low to high molecular weight.

3.5. Casework studies

3.5.1. Simulated casework

A total of 43 case stains and 18 reference samples were profiled. The results are shown in Table 2. The profiles obtained were all closely inspected, with details of any mixtures recorded and interpreted. Details of any non-allelic peaks, stutter bands and n (or A) bands [4,13] were recorded to characterise the system using criteria of Gill et al. [5].

3.5.2. Pilot studies

Two pilot studies were carried out in operational DNA casework units. Casework samples that had previously been profiled with SGM were reamplified using the new system and the results compared. Although these samples had not been extracted using washed chelex, it was important to establish that results could be obtained using unwashed chelex in order to reanalyse old extracted casework samples in the future. In the first pilot, 167 samples that comprised of blood, cigarette butts, stamps, envelopes and chewing gum were profiled and the success rates compared to the original SGM results (Table 3).

The second pilot was completed in two stages. In the first stage, 227 samples were processed (155 case stains and 113 reference samples); 220 had previously given full SGM profiles. The second stage involved profiling 41 samples that comprised mixtures, partials and other anomalies (Fig. 4). The extracts had been prepared with unwashed chelex when previously profiled with SGM and the same extracts were used for this study. The samples had been stored for at least 6 months at −20°C between the SGM analysis and the actual pilot study. With SGM Plus™, 27 of the initial 227 samples gave partial profiles and two gave no profiles.

Increased numbers of partial profiles were observed with SGM Plus™ (see Table 3 and Fig. 4) which was expected in light of the information obtained from the extraction optimisation experiments occurring concurrently. On inspection, the partial profiles had similar morphology to the patterns observed with inhibition or degradation of the DNA when unwashed chelex was used for extraction [11].

Table 2
Success rates of the crime stains from the simulated casework validation using AMP*Fl*STR® SGM Plus™[a]

Body Fluid	Number of samples	Full	Partial	Fail
Semen	12	9	1	2
Blood	15	12	2	1
Saliva	4	3	1	
Epithelial	5	3	1	1
Asperimic semen	4	2	2	–
Reference	18	18	–	–
Saliva/semen mixture	1		1	–
Saliva/blood mixture	2	2	–	–

[a] All of the 18 reference blood samples gave full profiles.

Table 3
The success rates for SGM Plus™ are comparable to the SGM results[a]

	System	No. of samples	Full profiles[b]	Partial[b]	Mixtures[b]	No result[b]	Species, no DNA[b]
Blood	SGM	89	77	3	1	5	3
			(86.6)	(3.3)	(1.1)	(5.6)	(3.4)
	SGM Plus	89	75	8	1	3	2
			(84.3)	(9)	(1.1)	(3.4)	(2.2)
Cigarette butts	SGM	60	43	6	3	5	3
			(71.7)	(10)	(5)	(8.3)	(5)
	SGM Plus	60	44	6	6	1	3
			(73.3)	(10)	(10)	(1.7)	(5)
Stamp, chewing gum, envelope	SGM	18	5	2	1	10	0
			(27.7)	(11.2)	(5.6)	(55.5)	(0)
	SGM Plus	18	5	4	0	9	0
			(27.7)	(22.3)	(0)	(50)	(0)

[a] There are fewer samples giving no result with the new system suggesting an apparent increase in sensitivity compared to the SGM system.
[b] The figures in parentheses are percentages.

When the genotypes were compared between the two systems, one sample showed a typing conflict for the HUMFIBRA locus. The crime stain and reference samples were designated as 19, 26 with SGM whereas the SGM Plus™ results showed only a 19 allele in the crime stain and one of the reference samples. The duplicate reference sample showed a distinct 19 peak and a peak close to the background level in the position of the 26 allele (95% peak area difference). When the SGM results were scrutinised more closely, it appeared that there was some imbalance at HUMFIBRA, with up to a 51% peak area difference in the SGM crime stain. This was probably caused by a point mutation in a primer binding site affecting SGM Plus™ and SGM. This is a rare event.

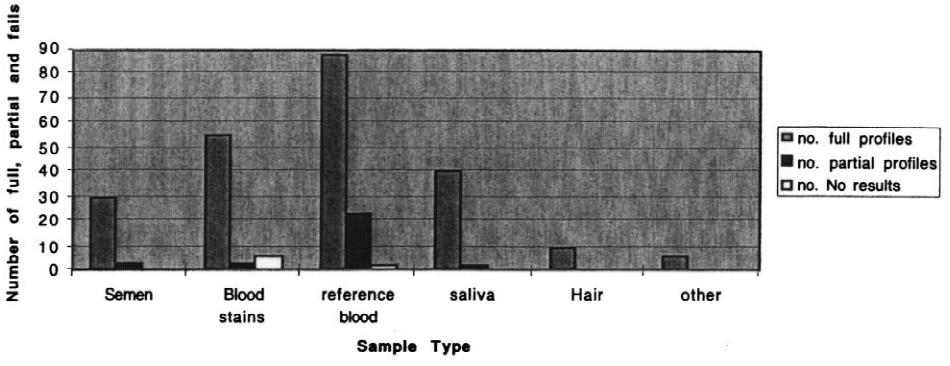

Fig. 4. Success rates with SGM Plus™ of each different sample type. Reference blood samples are the stains prepared from fresh liquid blood samples obtained from people involved in the investigation of the crime (includes both victims and potential suspects). Partial profiles are defined as samples where any locus has dropped out.

Walsh et al. [18] compared results from 1483 individuals profiled with SGM Plus™ and different multiplex primers and showed that just two alleles were found to be discordant at HUMVWF31/A. In addition, we carried out a concordance study of 1000 individuals to compare SGM with SGM Plus™ designations. No differences in typing were discovered (unpublished).

4. Conclusion

In comparison to previously reported STR systems used by the Forensic Science Service [7,10,12,13,20] the AMP*Fl*STR® SGM Plus™ gives greatly improved discrimination with an apparent increase in sensitivity. This is advantageous when dealing with smaller quantities of DNA. It was found necessary to modify the extraction method by introducing washed chelex into the protocol to minimise inhibition/degradation of the DNA and improve the quality of the resulting profile.

The primer sequences are different between SGM plus and SGM loci (except amelogenin) and hence a mutation affecting one primer binding site may not affect the primer site at the alternative system. This could result in one system being typed homozygote at a locus, due to a null allele, whilst the other showed a heterozygote. This is only relevant if samples processed with SGM are compared to those processed on SGM plus. Any differences can be easily resolved by repeating the sample with the alternative multiplex system.

In summary, the AMP*Fl*STR® SGM Plus™ multiplex appeared to be robust and reliable when applied to samples commonly found in a casework environment. It was an improvement on the SGM system previously used for casework and DNA database samples and contains all of the Interpol and ENFSI [5] recommended European loci.

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