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Variability in the detection of mixed profiles in four commercial autosomic STR multiplexes

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Abstract. Allelic mixtures cannot be detected in some evidences depending on the commercial kit used. The variability in the detection of mixed profiles with four multiplexes of a same manufacturer in the same DNA extracts of casework samples was analysed. SGMPlus™ has showed the best results in allelic mixture detection and Identifiler™ has showed to be the less useful. The criteria to determine if results have sufficient intensity and quality for mixture interpretation should be included in the validation process in the laboratories and, if necessary, to define the best multiplexes in typing casework samples to improve mixture detection. © 2005 Published by Elsevier B.V.

Keywords: Mixed STR profile; Allelic mixture; Forensic casework; DNA mixture detection

1. Introduction

The intensity of alleles observed at different genetic loci in mixed stains should depend on the characteristics of the sample, on the adaptations of each laboratory user to the manufacturer's instructions and, as recently proposed, on intrinsic differences of the commercial multiplexes [1]. Recommendations about typing differences due to allele dropout, in reference samples, were proposed from a concordance study with different kits from different manufacturers [2]. In this study, we analyse the variability in the detection of mixed profiles in casework evidences, by using four multiplexes of a same manufacturer in the same DNA extracts.

2. Material and methods

DNA extracts of 55 non-probative mixed evidences, selected from samples included in a previous study [3], were once amplified by AmpF*l*STR® Profiler Plus™ (Pro+),

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Table 1
Characteristics of the commercial kits in the detection of mixtures in 55 casework of non-probative samples[a]

Commercial kit	Mixture detection[b]	Full profiles	Allelic dropouts	Locus dropouts
SGMPlus™	96	87	60	11[c]
Profiler Plus™	96	82	60	18
Cofiler™	90	87	27	12
Identifiler™	89	84	84	35[c]

[a] Only peaks over 100 RFUs have been considered; data are given in percentages, $N=55$.
[b] At least one marker resulting with at least 3 alleles.
[c] One DNA extract did not give any result, $N=54$.

Cofiler™ (Co), Identifiler™ (Idf) and SGMPlus™ (SGM+ in the text) kits (Applied Biosystems), according to the manufacturers' instructions. The DNA extracts had been stored at −20 °C during a period of 5 to 10 years. All data were analysed with Genotyper® NT and GeneScan® NT Analysis software. Allele peaks were interpreted over 100 RFUs.

3. Results

STR profiles were concordant between them and with those of the corresponding previously typed samples from the same case; eleven samples were mixtures composed of more than two DNA sources. DNA mixture was lost in one sample and 3 of 55 samples yielded partial mixed profiles for the four kits. One DNA extract did not yield results when amplified with Idf and another sample with SGM+. Only 5 of 55 mixed profiles showed identical allelic designation over 100 RFUs for all markers; nevertheless, in most samples, alleles below the chosen signal intensity were present, although not considered. SGM+ and Pro+ showed the same higher rate of mixture detection, but the first one resulted in a higher percentage of full profiles and a lower of locus dropouts (Table 1). Idf has yield the worst results under most analysed parameters.

The incidence of allelic dropouts has been related to the number of genetic markers in each kit. Locus dropout were observed for all kits in the same sample six times for D2, five for D7, four for CSF and two times for D13 and D18 markers; the average of allelic dropouts in D2, D7 and CSF were also higher than the rest of the STR analysed (Table 2). Concordance in allele designation was

Table 2
Characteristics of the STR in the detection of mixtures in 55 casework of non-probative samples[a]

Marker[b]	VWA	D8	FGA	THO1	D3	D21	D19	D18	D5	D16	TPOX	D13	D7	CSF	D2
Mixture detection[c]	48	47	46	45	42	41	39	37	33	33	27	25	24	23	22
Allelic concordances[d]	30	32	24	35	32	23	**41**	28	**45**	29	38	35	25	29	33
Allelic dropouts	22	23	27	20	22	29	11	21	8	19	12	15	21	17	15
Locus dropouts[e]	1	–	6	2	1	4	–	9	1	5	3	6	**15**	**12**	**11**

[a] Only peaks over 100 RFUs have been considered.
[b] VWA(3); D8=D8S1179(3); FGA(3); D3=D3S1358(4); D21=D21S11(3); D19=D19S433(2); D18=D18S51(3); D5=D5S818(2); D16=D16S539(3); D13=D13S317(2); THO1(3); TPOX(2), D7=D7S820(3); CSF=CFS1PO(2); D2=D2S1338(2); numbers in brackets represent the number of kits containing each STR.
[c] At least 3 allele detections.
[d] The same allelic pattern over 100 RFUs in all samples.
[e] Markers included in the two samples that gave no results for Idf and for SGM+ have been not considered.

higher in D5 and D19, both markers are in first position in their fluorochrome groups and they are present in only two kits.

The analysis by the different position of the STRs in each fluorochrome group in each kit support our previous observations [3], with average detections of 35 mixed samples in first position, 32 in second, 26 in third, 17 in fourth and 13 in fifth (only D2 in Idf); the analysis by fluorochromes showed than STRs labelled in yellow yielded a slightly lower incidence in the detection of mixtures (51%) than the other colours (red and green 56% and blue 55% average of samples with at least three alleles over 100 RFUs). The presence/absence of some markers seems to be an important factor in the capacity of mixture detection for a kit. Greater averages of mixtures were found in two markers absent in Co: VWA and D8 (Table 2). The lower detection of mixtures was observed in D2, CSF, D7, D13 and TPOX; from them, only D2 is present in SGM+. The higher number of locus dropouts was detected in D7, which represents 9% of locus dropouts in Co, 18% in Pro+ and 25% in Idf.

4. Discussion

Primer sequences from Idf are the same as those included in Pro+ and Co kits (Identifiler User's Manual, A.B.); the SGM+ kit has incorporated some changes to improve the sensitivity [4]. Because in this study all analyses process have been performed by the same person, the characteristics of the DNA templates, the typing process and the interpretation of mixed profiles, could be eliminated as variables interfering in the results obtained with the four multiplexes. Only stochastic variations associated with sampling and those related to the improvement of the SGM+ kit should be added to the proper characteristics of the markers, and their combinations in the fluorochromes and positions. We have not considered all peaks detected below 100 RFUs, although increasing differences between the profiles established with the four kits used. In general, SGM+ has been the multiplex that showed the best results in allelic mixtures detection in our casework DNA extracts; Idf has been the worst. Since it consumes time and money, the reference samples matching evidentiary samples can be typed with one multiplex, but some evidences must be analyzed by duplicate with two different multiplexes, resulting in the confirmation of the results upon some markers. We consider that the use of Idf should be useful in typing reference samples and in those samples with mixed DNA, which should be interesting to eliminate the minor components in some markers. In conclusion, allelic mixtures cannot be detected in some evidences depending on the commercial kit used. The criteria to determine if results have sufficient intensity and quality for mixture detection should be included in the validation process in the laboratories and, if necessary, to define the best multiplexes in typing casework samples to improve mixture detection.

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