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2004–2005 GEP proficiency testing programs: Special emphasis on the interlaboratory analysis of mixed stains

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Abstract. The 2004–2005 GEP proficiency testing programs consisted of a simulated paternity case and a simulated forensic criminal case each including 3–5 reference samples (saliva or blood) and 2 forensic samples (mixed stains and clean or contaminated hair shafts). In the 2004 forensic test a mixture stain was analysed and apparently inconsistent results were observed between autosomal STR profiling and mitochondrial DNA sequencing results. In 2005, the forensic challenge was an unbalanced mixture stain of saliva and blood from two related contributors (sharing maternal and paternal lineages). Due to the stain characteristics, no lab detected the minor component in the mixture. This evidences the fact that the detection of a minor contributor in a mixture is still a key outstanding in forensic investigation. Also hair shafts contaminated with blood have been sent to be analysed and the results showed the influence of the extraction procedures applied.

Keywords: Mixed stain; Proficiency testing program; Hair analysis

1. Introduction

The GEP–ISFG proficiency testing program consists of simulated paternity and forensic criminal cases. Previous publication of the exercises results reported an overview of the program including the evaluation of possible causes of errors [1]. In this paper we would like to focus on the causes of errors of exercises 2004–2005 and the problems associated to DNA analysis in mixed stains. Other considerations related to these exercises results will be published elsewhere.

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2. Materials and methods

The 2004 exercise consisted of seven samples: 3 blood reference samples for a maternity test (M1–M3) and for the forensic case both a mixed stain: M6–100 μl saliva from a female, M4, and 50 μl of a 1:20 semen dilution (from M5) subsequently applied to a Whatman® Bloodstain Card–and 4 hair shafts (M7), with 2 blood reference samples (M4, M5).

The 2005 exercise included 4 reference samples (saliva from a child, M1, and blood M2–M4) for both the paternity and the forensic case. Forensic investigation consisted of the identification of a mixed stain (M5: 10 μl saliva and 30 μl blood from two related contributors M3 and M4, respectively) and the investigation of hair shafts: M6 (from M1 donor) contaminated with blood (from M4).

Exercises also included a theoretical paternity challenge test. All labs were asked to report the methods used including forensic preliminary analysis, the typing results of STRs (autosomal, X and Y STR) and mitochondrial DNA (mtDNA) as well as to interpret results including statistical evaluation. In the 2005 exercise electropherograms and analysis data were required.

3. Results and discussion

Current DNA analysis included in GEP–ISFG 2004 and 2005 exercises are listed at the table below. Only consensus markers are displayed. Consensus is obtained whenever a specific marker is analyzed at least by five labs and results being common in 70% of them provided the rest 30% an unequal result. More than 30 other markers not included in the table are reported by a lower number of laboratories.

Autosomal STRs			Y-STRs		
HUMFES/FPS	HUMLPL	D21S11	DYS 19	DYS 393	DYS 461 (GATA A7.2)
HUMTH01	ACTBP2(SE33)	D2S1338	DYS 385	DYS 437	GATA A10
HUMF13A01	D1S1656*	D3S1358	DYS 389 I	DYS 438	GATA C4 (DYS 635)
HUMVWA	D12S391	D5S818	DYS 389 II	DYS 458*	GATA H4
HUMTPOX	D13S317	D7S820	DYS 390	DYS 456*	DYS 439 (GATA A4)
HUMCSF1PO	D16S539	D8S1179	DYS 391	DYS 448*	DYS 460 (GATA A7.1)
HUMFIBRA/FGA	D18S51	Penta D	DYS 392		
HUMF13B	D19S433	Penta E			
Gender determination		AMELOGENIN	X-STR s		HUMPRTB

*Without consensus at 2004.

Discordances are still being made in the exercises, with a relevant incidence in the general results (see Table 1). The table shows how results obtained with manual techniques revealed higher error rate; those laboratories are continuously decreasing (26 labs at 2004 to 19 at 2005 exercise).

Table 1
Details of total number of determinations for STR profiling and error rate (%) observed at 2004 and 2005 exercises, depending on the detection procedure (manual/automatic)

	Manual				Automatic		
	System	*n*	Error	(%)	*n*	Error	(%)
2004 EXERCISE	Autosomic	905	26	2.8729	5299	68	1.282
Participating labs: 93	STRY	212	9	4.2453	1644	18	1.1029
Number of samples: 7	Others	99	5	5.0505	335	3	0.8955
Total determinations: 8494	Total	1216	40	3.2894	7255	89	1.2267
2005 EXERCISE	Autosomic	814	34	4.1769	6275	21	0.3347
Participating labs: 102	STRY	248	15	6.0484	2299	15	0.6525
Number of samples: 6	Others	72	1	1.3889	386	4	1.0363
Total determinations: 10094	Total	1134	50	4.4092	8960	40	0.4464

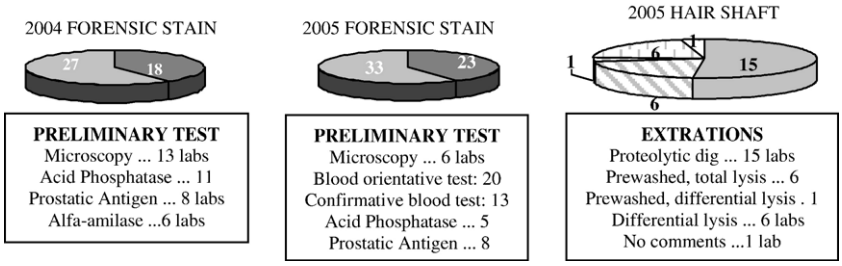

Fig. 1. Number of laboratories performing preliminary tests with details of the techniques used for forensic stains analysis and hair shafts extraction protocols.

Moreover the forensic tests have become the more fruitful of the exercises. Preliminary tests are not always performed (see Fig. 1): for the 2004 Forensic stain 18/45 laboratories performed preliminary tests while 27 labs proceeded only with genetic analysis. When analyzing this mixed stain for STR profiling, sample management errors, transcription errors and missing a contributor in the mix were observed. 73% of labs (33/45) got the correct answer (mixed stain contained both M4 and M5 contributors), 16% of labs did not detect the victim (7/45) and the rest of labs excluded the suspect contribution (4/45). Surprisingly a different result was obtained when some of the labs also studied this sample by mtDNA sequencing: 14 labs detected only the victim contribution in the mixed stain (previously detected as minor component) while 6 other labs observed different kinds of mixed mtDNA profiles (see Ref. [2]). Additional validation studies were planned by the GEP Working Group [3] to progress in the interpretation of mtDNA from different mixed stains of semen and another body fluids to investigate if the small number of mitochondria found in sperm could explain the mtDNA results observed by GEP participating labs.

In the 2005 exercise the mixed stain was analyzed by 56 laboratories, 24 of them describing preliminary tests (see Fig. 1). No lab detected the saliva component in the mixture due to its dilution with regards to the blood component (with an estimated DNA proportion of 1:100). This evidences the fact that the detection of a minor contributor in a mixture is still a key outstanding in forensic investigation.

Related to the mtDNA hair analysis, the 2004 results showed 73% of the lab answering correctly: hair shafts belongs to M5 contributor, also a 14% additional with a partial (HV 1 or HV 2) correct response. More discussion on the 2005 results (obtained from a hair shaft fragment contaminated with blood) is generated depending on the extraction procedures applied at each laboratory and its influence in final mtDNA results (see Fig. 1). Different treatments were performed to obtain mtDNA sequencing results. Better results were obtained whenever adequate treatment preceded analysis. It was more probable to obtain a mixed haplotype when a total lysis is performed while prewashing techniques and/or differential lysis gave more probably two separated haplotypes. A total of 7 of these labs detected only the blood contaminating the shafts.

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