

Table S1. General Aspects. Participants Characterization.

		GHEP-MIX6 (N = 25)	
		n (labs)	%
1. Kind of cases that your laboratory examine			
	Forensic	0	0.0
	Paternity	1	4.0
	Both	24	96.0
2. Send DNA profile to Criminal DataBase:			
	National	8	32.0
	Own	0	0.0
	Both	6	24.0
	No	10	40.0
	Do not report	1	4.0
3. Report results about mixture profiles of autosomal markers:			
	Yes	20	80.0
	No	2	8.0
	Only if there is reference sample	3	12.0
4. In case of obtaining a mixture profiles:			
	They are sent to Criminal DataBases	1	4.0
	They are not sent to Criminal DataBases	5	20.0
	They are only registered on the reports	6	24.0
	They are registered on both reports and Criminal DataBases	12	48.0
	Do not report	1	4.0
5. When mixture profiles are interpreted, allelic assignment has been carried out by			
	Automatic	0	0.0
	Manual	1	4.0
	Both	24	96.0
6. Your laboratory's mixture interpretation criterias have been validated:			
	Yes	11	44.0
	No	5	20.0
	Validation in progress	9	36.0

Table S2. Technical Aspects. Edition Tools.

		GHEP-MIX6 (N = 25)	
		n (labs)	%
7. Which edition software have you used:			
	GeneMapper	23	92.0
	GeneScan	0	0.0
	Genotyper	1	4.0
	PeakScanner	0	0.0
	Grape	1	4.0
8. Which criterias do you use to define a profile as a mixture:			
	≥ 4 STRs with ≥ 3 alleles	1	4.0
	≥ 3 STRs with ≥ 3 alleles	0	0.0
	≥ 2 STRs with ≥ 3 alleles	7	28.0
	Alleles Imbalance	0	0.0
	Amelogenin Imbalance (exclusively)	0	0.0
	≥ 4 STRs with ≥ 3 alleles and Alleles Imbalance	0	0.0
	≥ 3 STRs with ≥ 3 alleles and Alleles Imbalance	4	16.0
	≥ 2 STRs with ≥ 3 alleles and Alleles Imbalance	13	52.0
	≥ 3 STRs with ≥ 3 alleles and Amelogenin Imbalance	0	0.0
	≥ 2 STRs with ≥ 3 alleles and Amelogenin Imbalance	0	0.0
	Others	0	0.0
9. In the present exercise, the alleles have been assigned when the peak is higher than:			
	150 RFUs	3	12.0
	100 RFUs	1	4.0
	50 RFUs	19	76.0
	Others: 40 RFUs	2	8.0

Table S2. Technical Aspects. Edition Tools.

		GHEP-MIX6 (N = 25)	
		n (labs)	%
10. The "stutter" positions (n-4/n+4) have been assigned as possible alleles:			
	Every	1	4.0
	> 15% from principal allele	0	0.0
	> 150 RFUs	0	0.0
	> 100 RFUs	0	0.0
	> 50 RFUs	0	0.0
	The assignment has been variable depending on which STR marker is taken into account	10	40.0
	> 15% from principal allele and > 150 RFUs	0	0.0
	> 15% from principal allele and > 100 RFUs	1	4.0
	> 15% from principal allele and > 50 RFUs	1	4.0
	The assignment has been variable depending on which STR marker is taken into account, and > 150 RFUs	2	8.0
	The assignment has been variable depending on which STR marker is taken into account, and > 100 RFUs	0	0.0
	The assignment has been variable depending on which STR marker is taken into account, and > 50 RFUs	10	40.0
	The assignment has been variable depending on which STR marker is taken into account, and > 15% from principal allele	0	0.0
11. Which is / are the principal obstacle/s that your laboratory have to face up to interpret a mixture profiles?			
	Lack of an unique criterion on the laboratory	5	20.0
	Lack of training	3	12.0
	Lack of an unique criterion on the laboratory and Lack of training	6	24.0
	There are not any obstacle	0	0.0
	Others	11	44.0
11.1. Others obstacles to face up to interpret a mixture profiles...			
	Nature of the sample	5	20.0
	Combination of factors	5	20.0
	Validation of methods	1	4.0

Table S3. Statistical Aspects.

		GHEP-MIX6 (N = 25)	
		n (labs)	%
12. Using profiles: Sample 1			
Inclusion		5	20.0
Exclusion		3	12.0
Both		17	68.0
12. Using profiles: Sample 2			
Inclusion		4	16.0
Exclusion		4	16.0
Both		17	68.0
13. LR calculation: Sample 1			
Yes		7	28.0
No		6	24.0
Yes, with conditions		12	48.0
13. LR calculation: Sample 2			
Yes		7	28.0
No		13	52.0
Yes, with conditions		5	20.0
14. LR calculation is carried out by using:			
Automatic (software)		22	88.0
Manual		1	4.0
Both		2	8.0
14.1. Used software			
DNAMIX		7	15.2
BDGen		1	2.2
LRmix_R		0	0.0
LRmixStudio		16	34.8
GFF		4	8.7
Lrmezpcr		5	10.9
Carralero (2006)		1	2.2
Grape		1	2.2
CODIS		1	2.2
LIMS		1	2.2
Own calculation sheer		6	13.0
YHRD		2	4.3

GHEPMIX6 – Profiles Results

Mixture of Autosomal Markers

Expected Profile of Autosomal Markers

loci	D10S1248	vWA	D16S539	D2S1338	AMEL	D8S1179	D21S11	D18S51	D22S1045	D19S433	TH01	FGA	D2S441	D3S1358	D1S1656	D12S391
mixture	13, 14, 15, 16, 17	14, 16, 17, 18	9, 12, 13	17, 18, 19, 20, 22, 24	X, Y	12, 13, 15	29, 31.2, 32.2	11, 15, 16, 17	15, 16	13, 14, 15, 16	6, 7, 8, 9.3	21, 22, 24, 25	10, 11, 11.3, 14	14, 15, 16, 17, 18	15, 15.3, 16.3, 17, 18.3	19, 19.3, 20, 21, 25

Labs Results for Mixture of Autosomal Markers

# lab	D10S1248	vWA	D16S539	D2S1338	AMEL	D8S1179	D21S11	D18S51	D22S1045	D19S433	TH01	FGA	D2S441	D3S1358	D1S1656	D12S391
GM2015.01																
GM2015.02																
GM2015.03																
GM2015.05																
GM2015.06																
GM2015.07																
GM2015.08																
GM2015.09																
GM2015.10																
GM2015.11																
GM2015.12																
GM2015.13																
GM2015.14																
GM2015.15																
GM2015.16																
GM2015.17																
GM2015.18																
GM2015.19																
GM2015.20																
GM2015.21																
GM2015.22																
GM2015.23																
GM2015.24																
GM2015.26																
GM2015.27																

	Correct Alleles
	Stutters Included (signaled)
	Drop-out / Drop-in

GHEPMIX6 – Profiles Results

Mixture of Y-chromosome Markers

Expected Profile of Y-chromosome Markers

loci	DYS456	DYS389I	DYS390	DYS389II	DYS458	DYS19	DYS385	DYS393	DYS391	DYS439	DYS635	DYS392	GATAHA.1(H4)	DYS437	DYS438	DYS448
mixture	15, 16	13	24	29, 30	17, 18	13, 14	12, 14, 15, 16	13, 15	11	11, 12	22, 23	13, 14	20, 21	14	8, 12	18, 21

Labs Results for Mixture of Y-chromosome Markers

results types	DYS456	DYS389I	DYS390	DYS389II	DYS458	DYS19	DYS385	DYS393	DYS391	DYS439	DYS635	DYS392	GATAHA.1(H4)	DYS437	DYS438	DYS448	n	%	Comments
1	15, 16	13	24	29, 30	17, 18	13, 14	12, 14, 15, 16	13, 15	11	11, 12	22, 23	13, 14	20, 21	14	8, 12	18, 21	12	48%	
2	15, 16	13	24	29, 30	17, 18	13, 14	12, 14, 15, 16	13, 15	11	11, 12	22, 23	13, 14	20(11), 21(12)	14	8, 12	18, 21	2	8%	
3	15, 16	13	24	29, 30	17, 18	13, 14	12, 14, 15, 16	13, 15	11	11, 12	22, 23	13, 14	11, 12	14	8, 12	18, 21	7	28%	
4	15, 16	13	24	29, 30	17, 18	13, 14	12, 14, 15, 16	13, 15	11	11, 12	22, 23	13, 14	20, 21	14	8, 12	18*, 21	1	4%	(*): "allele slightly below the stochastic threshold"
5	15, 16'	13	24	29', 30	17', 18	13, 14'	12', 14', 15, 16	13', 15	11	11, 12'	22, 23'	13', 14	20, 21'	14	8, 12'	18', 21	1	4%	('): "alleles of the majority profile"
6	15, m16	13	24	30, m29	18, m17	13, m14	15, 16, m12, m14	15, m13	11	11, m12	22, m23	14, m13	20, m21	14	8, m12	21, m18	1	4%	(m): "minority component"
7	15, [16]	13	24	[29], 30	[17], 18	13, [14]	[12], [14], 15, 16	[13], 15	11	11, [12]	22, [23]	[13], 14	20, [21]	14	8, [12]	[18], 21	1	4%	[]: "alleles detected at lower intensity"
																	25	100%	