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GRUPO DE HABLA ESPAÑOLA Y PORTUGUESA DE LA ISFG

GRUPO DE LÍNGUAS ESPANHOLA E PORTUGUESA DA ISFG

Instituto Nacional de Toxicología
y Ciencias Forenses

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INTERCOMPARISON PROGRAM
“ANALYSIS OF DNA POLYMORPHISMS IN BLOODSTAINS AND OTHER BIOLOGICAL SAMPLES”

BASIC LEVEL ***EXERCISE EIADN -33 (2025)*** ***DEADLINE: 15/05/2025***

Items sent

2025/Kinship Module
M1 to M3: reference items
2025/Forensic module
M4: forensic unknown item
M5: hair sample

Seal number

Approach:

2025/Kinship Module – Basic level

Practical Kinship study

- **M1, M2, M3:** reference items for genetic profiling.

Theoretical Kinship study

Participants are asked to solve the proposed theoretical study.

2025/Forensic Module – Basic level

Practical Forensic study

- **M4:** forensic item for genetic profiling.
- **M5:** hair for mitochondrial DNA analysis.

- Determine the body fluid component or possible components of the item M4.
- Could any of the donors from the reference items M1, M2, M3 have contributed to the item M4?

Theoretical Forensic study

Participants are asked to solve the proposed theoretical study.

Methodology to be used

The analyses will be performed by using the markers and methods chosen by the laboratory or those use of routine or that are being under implementation. The items must be processed as real casework and, if possible, as blind samples.

The National Institute of Toxicology and Forensic sciences is the accreditation holder
Activities marked are not bound to ENAC accreditation

ENAC
ISO 17043
Nº8 / PPI016

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1. **Methodology**

2. **Practical studies results**

3. **Practical studies conclusions**

4. **Theoretical studies**

1. Methodology *Read carefully the instructions provided before filling in this section*

1.1 DNA Extraction, purification/concentration and quantitation

TABLE 1

Item	Differential lysis (Yes/No)	Extraction Purification/ Concentration (Code)	EP00 (Specify)	Quantitation (Code)	C00 (Specify)
M1					
M2					
M3					
M4					
M5					

See Appendix 2025 for codes

1.2 STRs methodology

1.2.1 Multiplex kits methodology

TABLE 2A (Multiplex kits)

If a kit not included in the table is used, add it in the last rows.

Multiplex	Report 'YES' if used	Detection (Code)	D00 (Specify)
FFFL (Promega)			
PowerPlex 16/16 HS (Promega)			
PowerPlex ESI 16 (Promega)			
PowerPlex ESX 16 (Promega)			
PowerPlex ESI 17 (Promega)			
PowerPlex ESX 17 (Promega)			
PowerPlex 18D (Promega)			
Profiler Plus (AB)			
SGM Plus (AB)			
Identifiler (AB)			
Identifiler Plus (AB)			
Identifiler Direct (AB)			
NGM (AB)			
NGM SElect (AB)			
MiniFiler (AB)			
Investigator ESSplex (Qiagen)			
Investigator ESSplex SE (Qiagen)			
Investigator IDplex (Qiagen)			
YFiler (AB)			
PowerPlex Y (Promega)			
Argus X-8 (Biotype)			
Investigator Argus X-12 (Qiagen)			
XSTR-Decaplex GHEP (Gusmão)			
PowerPlex CS7 (Promega)			
Profiler (AB)			
Investigator Argus Y-12 (Qiagen)			
SEfiler (AB)			
PowerPlex 23Y(Promega)			
PowerPlex Fusion System (Promega)			
Global Filer (AB)			
PowerPlex 21 (Promega)			
Investigador 24plex QS (Qiagen)			

Multiplex	Report 'YES' if used	Detection (Code)	D00 (Specify)
PowerPlex Fusion 6C System (Promega)			
Verifiler (AB)			
YFiler Plus (AB)			
Investigator ESSplex plus (Qiagen)			
Investigator ESSplex Plus (Qiagen)SE			
Investigator IDplex Plus (Qiagen)			
Investigator HDplex (Qiagen)			
Investigator Argus X-12 QS (Qiagen)			

See Appendix 2025 for codes

1.2.2 Other methodology for autosomal STR markers and amelogenin

TABLE 2B

Fill in ONLY in case kits multiplex are not used or additional STR markers are utilized. Indicate the number of markers, the primers and the methodology used.

Number of markers	Primer/Ladder (Code)	PL00 (Specify)	Detection (Code)	D00 (Specify)

See Appendix 2025 for codes

1.2.3 Other methodology for Y-STR markers

TABLE 2C

Fill in ONLY in case kits multiplex are not used or additional Y-STR markers are utilized. Indicate the number of markers, the primers and the methodology used.

Number of markers	Primer/Ladder (Code)	PL00 (Specify)	Detection (Code)	D00 (Specify)

See Appendix 2025 for codes

1.2.4 Other methodology for X-STR markers

TABLE 2D

Fill in ONLY in case kits multiplex are not used or additional X-STR markers are utilized. Indicate the number of markers, the primers and the methodology used.

Number of markers	Primer/Ladder (Code)	PL00 (Specify)	Detection (Code)	D00 (Specify)

See Appendix 2025 for codes

1.3 Mitochondrial DNA methodology

1.3.1 Amplification parameters

TABLE 3

Report each primer set in one single box and name them according to the strand (L or H) and 3' nucleotide position (Ex. L15997/H00619)

Primers sets for amplification					Nr of cycles
Item	Forward/reverse	Forward/reverse	Forward/reverse	Forward/reverse	
M1-M3					
M4					
M5					

1.3.2 Sequencing and editing parameters

TABLE 4

Item	PU	QS	PE	S	SE
M1-M3					
M4					
M5					

See Appendix 2025 for codes

1.4 Methodology for body fluid identification of item M4

TABLE 5

If you have performed any test in order to confirm or investigate the presence of body fluids in the items M4, you must report the code for the used method and the obtained result (negative, positive or inconclusive). Please, in case that you report 'Other', specify.

Method (Code)	Other (Specify)	Results (Negative/Positive/Inconclusive)	Remarks

See Appendix 2025 for codes

1.5 Other considerations regarding methodology different to reported in the preceding tables

2. Practical studies results:

Read carefully the instructions provided in order to fill in the results tables and the rules of participation in order to know the establishment of assigned values and the evaluation of results https://ghep-isfg.org/en/proficiency/participation/

2.1 STRs Results

ALL PARTICIPANTS OF THE FORENSIC MODULE, MUST COMPLETE COMPULSORY THE COLUMN OF TOTAL ALLELES DETECTED REGARDLESS THE EXTRACTION SYSTEM USED. The 1st and 2nd fraction columns are additional and optional, in case the laboratory have performed differential lysis and want to reflect its result.

2.1.1 Autosomal STRs and amelogenin

TABLE 6A

KINSHIP MODULE				FORENSIC MODULE		
MARKER	M1	M2	M3	M4		
				Total of alleles detected Ex:9-11-15-17	1ST fraction Ex:9-17	2nd fraction Ex: 11-15
AMEL						
D8S1179						
D21S11						
D7S820						
CSF1PO						
D3S1358						
TH01						
D13S317						
D16S539						
D2S1338						
D19S433						
vWA						
TPOX						
D18S51						
D5S818						
FGA						
Penta D						
Penta E						
D10S1248						
D22S1045						
D2S441						
D1S1656						
D12S391						
SE33						
FES/FPS						
F13A01						
F13B						
LPL						
Penta C						
D6S1043						

2.1.2 Y-STRs

TABLE 6B

KINSHIP MODULE				FORENSIC MODULE		
MARKER	M1	M2	M3	M4		
				Total of alleles detected Ex: 13-15	1^{ST} fraction Ex: 15	2^{nd} fraction Ex: 13
DYS456						
DYS389 I						
DYS390						
DYS389 II						
DYS458						
DYS19						
DYS385						
DYS393						
DYS391						
DYS439 (GATA A4)						
DYS635 (GATA C4)						
DYS392						
GATAH4						
DYS437						
DYS438						
DYS448						
DYS460 (GATA A7.1)						
DYS461 (GATA A7.2)						
GATAA10						
DYS388						
DYS576						
DYS481						
DYS549						
DYS533						
DYS570						
DYS643						
DYS627						
DYS518						
DYS449						
DYF387S1						

2.1.3 X-STRs

TABLE 6C

KINSHIP MODULE				FORENSIC MODULE		
				M4		
MARKER	**M1**	**M2**	**M3**	**Total of alleles detected Ex: 12-15-17-20**	**1ST fraction Ex: 12-15**	**2nd fraction Ex: 17-20**
HPRTB						
DXS8378						
DXS9898						
DXS7133						
GATA31E08						
GATA172D05						
DXS7423						
DXS6809						
DXS7132						
DXS9902						
DXS6789						
DXS10103						
DXS10134						
DXS10074						
DXS10101						
DXS10135						
DXS10146						
DXS10079						
DXS10148						

2.2 Mitochondrial DNA results

In Table **7A, report the initial and final positions of the edited regions** and in **Table 7B report the haplotypes in the order requested in the instructions**

TABLE 7A

ITEMS		EDITED REGIONS
KINSHIP MODULE		
M1		
M2		
M3		
FORENSIC MODULE		
M4	**1st fraction**	
	2nd fraction	
Hair M5		

TABLE 7B

ITEMS		HAPLOTYPE
KINSHIP MODULE		
M1		
M2		
M3		
FORENSIC MODULE		
M4	**1st fraction**	
	2nd fraction	
Hair M5		

3.Practical Studies Conclusions

3.1 Kinship Module

3.1.1 *Remarks about items M1, M2 and M3

Indicate any comments or remarks, you consider, about the analyzed items. Please, remember that only the genetic profiling of the reference items M1 to M3 is required; it is not necessary to investigate a genetic relationship among them.

3.2 Forensic Module

3.2.1 Determine the body fluid component or possible components of the item M4.

Components (mark with an X the component/s detected)

	Blood	Semen	Saliva
M4	☐	☐	☐

3.2.2 Indicate the minimum number of contributors detected in the item M4.

	1	2	3
M4	☐	☐	☐

3.2.3 Could any of the donors from the reference items M1, M2, M3 have contributed to the item M4?

	M1	M2	M3
M4	☐	☐	☐

3.2.4 *Remarks about items M4 and M5.

4. Theoretical studies

Read carefully the instructions provided in order to fill in the results tables and the rules of participation in order to know the establishment of assigned values and the evaluation of results
https://ghep-isfg.org/en/proficiency/participation/

In order to solve the theoretical studies (kinship and forensic) it is assumed that:

- the population is in Hardy-Weinberg equilibrium and that no correction is made due to population substructure (theta=0).
- silent alleles rate and mutation rate are 0.
-drop in, drop out correction=0

Calculations have to be made by using the "*2025 Alleles Frequencies*" table provided.

4.1 Kinship theoretical study

4.1.1 Approach

A fatal accident occurs. A tanker truck carrying flammable material runs off the road and falls down an embankment, subsequently catching fire. Because of the load it was carrying, the possible owner of the tanker is located. The driver is believed to be a man named Fernando who used to cover that route and who does not answer his parents´calls. The body has been completely burned and the only way to identify it is through DNA analysis.

The genetic profile of Fernando's biological parents is available: Paco and Dolores.

o The couple will be considered to have had only one son: Fernando.
o There is no doubt that Paco and Dolores are Fernando's biological parents.

Marker	Victim	Dolores	Paco
D8S1179	13- 14	13- 14	13- 14
D21S11	28- 32.2	30.2- 32.2	28
D7S820	11	10- 11	10- 11
CSF1PO	11	11	11
D3S1358	15- 16	15- 18	14- 16
TH01	8- 9.3	6- 9.3	6- 8
D13S317	11	10- 11	10- 11
D16S539	11- 12	10- 12	11- 13
D2S1338	19- 25	17- 19	20- 25
D19S433	14- 15.2	13- 15.2	14- 15
VWA	17- 18	17- 18	15- 17
TPOX	8- 9	8- 11	9- 11
D18S51	12- 16	11- 12	13- 16
D5S818	11- 13	9- 11	10- 13
FGA	22- 24	20- 24	22
D10S1248	15	12- 15	15
D1S1656	16- 17.3	15- 16	12- 17.3
D22S1045	15- 16	15- 17	16
D2S441	11	10- 11	10- 11
D12S391	18	18	18- 22
PENTA_D	9- 12	9	12- 13
PENTA_E	12- 15	7- 15	12- 17
D6S1043	11- 12	11- 20	12- 14
Amelogenin	X-Y	X	X-Y

4.1.2. **Paternity-maternity index**

Calculate the paternity-maternity index taking into account the following hypotheses:

H0	The burned body is the son of Paco and Dolores
H1	The burned body is the son of a man and a woman not genetically related to each other or to Paco and Dolores

Report the partial paternity-maternity indexes and the total PMI in **Table 8**.

Use scientific notation (Excel format) and rounding off to 4 decimals places Ex. 1,2346E-01

TABLE 8

Markers	PMI
D8S1179	
D21S11	
D7S820	
CSF1PO	
D3S1358	
TH01	
D13S317	
D16S539	
D2S1338	
D19S433	
VWA	
TPOX	
D18S51	
D5S818	
FGA	
D10S1248	
D1S1656	
D22S1045	
D2S441	
D12S391	
PENTA_D	
PENTA_E	
D6S1043	
Total PMI	

4.1.3 Software/s used to carry out the statistical calculations.

Program	version	Remarks (other software, comments, etc)
Familias		
DNA view		
PatPCR		
BDGen		
PatCan		

Program	version	Remarks (other software, comments, etc)
Genética Forense Final		
Home-made Software		
Others[1]		

[1] If your software is not displayed in the table, chose “others” and specify it in the cell Remarks

4.1.4 Hand-made calculations. Formulas used

In the case, your laboratory performs all calculations by hand, then report the used formulas in **Table 9**

TABLE 9

Markers	KI
D8S1179	
D21S11	
D7S820	
CSF1PO	
D3S1358	
TH01	
D13S317	
D16S539	
D2S1338	
D19S433	
VWA	
TPOX	
D18S51	
D5S818	
FGA	
D10S1248	
D1S1656	
D22S1045	
D2S441	
D12S391	
PENTA_D	
PENTA_E	
D6S1043	
Total PMI	

4.1.5 *Conclusions and remarks about the kinship theoretical study.

4.2 Forensic theoretical study

4.2.1 Approach

A young woman reports a sexual assault 10 days after the incident. He leads the police to where the events took place and there they find panties, which the victim recognizes as hers.
The panties are analyzed and a mixed profile is obtained, which is entered into CODIS giving a match with the profile of a robbery suspect found in the database identified as RF1810024-EIADN-M.

The reference profile of the victim is available

Markers	Panties mixed Profile (M)	RF1810024-EIADN-M
D3S1358	15- 16	16
D1S1656	11- 13- 14- 18.3	11- 14
D2S441	11- 12- 14	12
D10S1248	12- 13- 14	12-14
D13S317	11- 12- 14	12
Penta E	11- 15	11- 15
D16S539	8- 10- 13	8- 13
D18S51	12- 13- 14- 16	12- 16
D2S1338	17- 21- 22	17- 22
CSF1PO	12- 13	12- 13
Penta D	9- 11- 13	11
TH01	6- 9	9
VWA	14- 16- 17- 20	16- 17
D21S11	28- 30- 32.2- 33.2	28- 32.2
D7S820	10- 11- 12	11
D5S818	10- 12	10- 12
TPOX	8- 9	8- 9
D8S1179	13- 14- 16	14
D12S391	17- 18- 19- 24	17- 18
D19S433	12- 13- 14.2	12- 14.2
SE33	19- 21- 30.2	19- 21
D22S1045	11- 14- 15- 16	14- 16
FGA	18- 21- 25	21- 25
D6S1043	17- 19- 20	17- 19
Amelogenin	X-Y	X-Y

4.2.2. LR value

Report the partial Likelihood Ratio (LR) values, as well as the total LR in **Table 10**, according to the following hypothesis:

H0	A woman and RF1810024-EIADN-M have contributed to the mixture of genetic profiles obtained in the panties.
H1	A woman and an unknown person taken at random from the population and not genetically related to the previous ones have contributed to the mixture.

TABLE 10

Use scientific notation (Excel format) and rounding off to 4 decimals places . Ex. 1,2346E-01

Markers	LR
D3S1358	
D1S1656	
D2S441	
D10S1248	
D13S317	
Penta E	
D16S539	
D18S51	
D2S1338	
CSF1PO	
Penta D	
TH01	
VWA	
D21S11	
D7S820	
D5S818	
TPOX	
D8S1179	
D12S391	
D19S433	
SE33	
D22S1045	
FGA	
D6S1043	
Total LR	

4.2.3 Software/s used to carry out the statistical calculations.

Program	version	Remarks (other software, comments, etc)
LRmix Studio		
LR mezcla v inteligente		
EuroForMix		

Program	version	Remarks (other software, comments, etc)
DNAMix		
Genética Forense Final		
Home-made Software		
DNA View		
Others[2]		

[2] *If your software is not displayed in the table, choose "others" and specify it in the cell "Remarks".*

4.2.4 Handmade calculations. Formulas used.

In case of only handmade calculations, then report the used formulas in **Table 11.**

TABLE 11

Markers	LR
D3S1358	
D1S1656	
D2S441	
D10S1248	
D13S317	
Penta E	
D16S539	
D18S51	
D2S1338	
CSF1PO	
Penta D	
TH01	
VWA	
D21S11	
D7S820	
D5S818	
TPOX	
D8S1179	
D12S391	
D19S433	
SE33	
D22S1045	
FGA	
D6S1043	
Total LR	

4.2.5 *Conclusions

Issue a conclusion regarding the results obtained.

Taking into account the reference sample of the victim. Answer the following questions:

Marker	**Victim (V)**
D3S1358	15
D1S1656	13- 18.3
D2S441	11- 14
D10S1248	13- 14
D13S317	11- 14
Penta E	11
D16S539	10- 13
D18S51	13- 14
D2S1338	21
CSF1PO	12- 13
Penta D	9- 13
TH01	6- 9
VWA	14- 20
D21S11	30- 33.2
D7S820	10- 12
D5S818	10- 12
TPOX	8- 9
D8S1179	13- 16
D12S391	19- 24
D19S433	13
SE33	21- 30.2
D22S1045	11- 15
FGA	18
D6S1043	20
Amelogenin	X

4.2.6 * Regarding the marker D1S1656, for which:

- The profile obtained from the mixture (M) is 11- 13- 14- 18.3-
- The **RF1810024-EIADN-M** 's profile (S) is 11-14, and
- The victim's profile (V) is 13-18.3,

Determine the Likelihood Ratio (LR)* for this marker under the following hypotheses:

H0	The victim (V) and RF1810024-EIADN-M contributed to the genetic mixed profile obtained from the underwear.
H1	The victim (V) and an unrelated random individual (D) from the population, not genetically related to the others, contributed to the mixture.

Marker	**LR**
D1S1656	

4.2.7*Regarding the previous item, indicate the formula used for calculating the Likelihood Ratio (LR) for that marker:

a) 1/(f11* f14)
b) 1/(f13 * f18.3)
c) 1/(2*f11* f14)
d) 1/(2* f13 * f18.3)
e) 1/(f11 * f13 * f14 * f18.3)

5. Remarks about this exercise

6. Suggestions for subsequent exercises

7.Compromises to be met by the participant

The analyses, both, the generated results and their statistical evaluation have been performed in the facilities of the participating laboratory and by its own staff, following working protocols used in routine casework together with safety precautions. **In accordance with the donors' consent, these items will be processed anonymously for the Intercomparison Exercise INTCFM/GHEP-ISFG. Additionally they could be used as a reference material and/or quality control for the laboratory either using the techniques required in the Exercise or other forensic techniques but always for the purpose of human identification, analyzing non coding regions or regions that would not provided sensitive information about the donor: illnesses, pathologies or other genetic information which could infringe his/her privacy.**

Name of the person in charge

Date and signature

WOULD YOU LIKE TO RECEIVE A CERTIFICATE OF PARTICIPATION?

Kinship Module **(Basic level)** (Yes/No)	**Forensic Module** **(Basic level)** (Yes/No)

CHOOSE THE LANGUAGE OF THE CERTIFICATE **SPANISH** ☐
ENGLISH ☐
BOTH ☐

Note.- In order to receive the certificate of participation you must return this form duly signed.