



GRUPO DE HABLA ESPAÑOLA Y PORTUGUESA DE LA ISFG

GRUPO DE LÍNGUAS ESPANHOLA E PORTUGUESA DA ISFG

MINISTERIO DE LA PRESIDENCIA JUSTICIA Y RELACIONES CON LAS CORTES

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## INTERCOMPARISON PROGRAM

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

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

Submitted items2025/Forensic module

M6: Forensic unknown item

M7: Forensic unknown item

M8: Forensic unknown item

Seal number

Approach:

## 2025/Forensic Module – Advanced level

**Practical Forensic study**

- **M6:** forensic unknown item for body fluid identification and genetic profiling
  - **M7:** forensic unknown item for body fluid identification and genetic profiling.
  - **M8:** forensic unknown item for body fluid identification and genetic profiling.
- ◆ Indicate the nature of the body fluids and the minimum number of contributors detected in the items M6, M7 and M8.
  - ◆ Could any of the donors from the reference items M1, M2, M3 have contributed to M6, M7 or M8?.

**Methodology to be used**

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

**INDEX**

	Page
<b>1. Methodology</b>	
<b>1.1.</b> DNA extraction, purification/concentration and quantitation	3
<b>1.2.</b> STRs methodology	
1.2.1. Multiplex kits methodology	3
1.2.2. Other methodology for autosomal STR markers and amelogenin	4
1.2.3. Other methodology for Y-STR markers	4
1.2.4. Other methodology for X-STR markers	4
<b>1.3.</b> Mitochondrial DNA methodology	
1.3.1. Amplification parameters	4
1.3.2. Sequencing and editing parameters	5
<b>1.4.</b> Methodology for body fluid identification of items M6, M7 and M8	5
<b>1.5.</b> Other considerations regarding meth different to reported in the preceding tables	5
<b>2. Practical studies results</b>	
<b>2.1.</b> STR markers results	
2.1.1. Autosomal STRs and amelogenin	6
2.1.2. Y-STRs	8
2.1.3. X-STRs	10
<b>2.2.</b> Mitochondrial DNA results	12
2.2.1 Remarks and conclusions about mitochondrial DNA results	12
<b>3. Practical studies conclusions</b>	
<b>3.1.</b> Forensic module	12
3.1.1 Question 1	12
3.1.2 Question 2	12
3.1.3 Question 3	13
3.1.4 Conclusions and remarks about items M6, M7	13
<b>4. Remarks about this exercise</b>	14
<b>5. Suggestions for subsequent exercises</b>	14
<b>6. Compromises to be met by the participant</b>	14
<b>Date and signature of the person in charge</b>	14
<b>Request for certification</b>	14

**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)
M6					
M7					
M8					

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)			
COfiler (AB)			
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 Fussion System (Promega)			
Global Filer (AB)			
PowerPlex21 (Promega)			
Investigator 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**

**TABLA 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.

Numbers of Makers	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 Marker s	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	
M6					
M7					
M8					

See Appendix 2025 for codes

**1.3.2 Sequencing and editing parameters**

**TABLE 4**

Item	PU	QS	PE	S	SE
M6					
M7					
M8					

See Appendix 2025 for codes

**1.4 Methodology for body fluid identification of items M6, M7 and M8**

**TABLE 5**

If you have performed any test in order to confirm or investigate the presence of body fluids in the items M6, M7 and M8 You must report the code for the used method and the obtained result (negative, positive or inconclusive). Please, in the case that you report 'Other', specify.

Item	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 MUST COMPLETE COMPULSORY THE COLUMN OF TOTAL ALLELES DETECTED REGARDLESS THE EXTRACTION SYSTEM USED.** The 1<sup>st</sup> and 2<sup>nd</sup> fraction columns are additional and optional, in case the laboratory have performed differential lisis and want to reflect its result.

**2.1.1 Autosomal STRs and amelogenin**

**TABLE 6A**

FORENSIC MODULE				
MARKER	ITEM	Total of alleles detected Ex: 9-11-13-15	1 <sup>st</sup> fraction Ex: 9-13	2 <sup>nd</sup> fraction Ex: 11-15
AMEL	M6			
D8S1179	M6			
D21S11	M6			
D7S820	M6			
CSF1PO	M6			
D3S1358	M6			
TH01	M6			
D13S317	M6			
D16S539	M6			
D2S1338	M6			
D19S433	M6			
vWA	M6			
TPOX	M6			
D18S51	M6			
D5S818	M6			
FGA	M6			
Penta D	M6			
Penta E	M6			
D10S1248	M6			
D22S1045	M6			
D2S441	M6			
D1S1656	M6			
D12S391	M6			
SE33 (ACTBP2)	M6			
FES/FPS	M6			
F13A01	M6			
F13B	M6			
LPL	M6			
Penta C	M6			
D6S1043	M6			
	M6			
AMEL	M7			
D8S1179	M7			
D21S11	M7			
D7S820	M7			
CSF1PO	M7			

FORENSIC MODULE				
MARKER	ITEM	Total of alleles detected Ex: 9-11-13-15	1 <sup>st</sup> fraction Ex: 9-13	2 <sup>nd</sup> fraction Ex: 11-15
D3S1358	M7			
TH01	M7			
D13S317	M7			
D16S539	M7			
D2S1338	M7			
D19S433	M7			
vWA	M7			
TPOX	M7			
D18S51	M7			
D5S818	M7			
FGA	M7			
Penta D	M7			
Penta E	M7			
D10S1248	M7			
D22S1045	M7			
D2S441	M7			
D1S1656	M7			
D12S391	M7			
SE33 (ACTBP2)	M7			
FES/FPS	M7			
F13A01	M7			
F13B	M7			
LPL	M7			
Penta C	M7			
D6S1043	M7			
	M7			
AMEL	M8			
D8S1179	M8			
D21S11	M8			
D7S820	M8			
CSF1PO	M8			
D3S1358	M8			
TH01	M8			
D13S317	M8			
D16S539	M8			
D2S1338	M8			
D19S433	M8			
vWA	M8			
TPOX	M8			
D18S51	M8			
D5S818	M8			
FGA	M8			
Penta D	M8			
Penta E	M8			
D10S1248	M8			
D22S1045	M8			
D2S441	M8			

FORENSIC MODULE				
MARKER	ITEM	Total of alleles detected Ex: 9-11-13-15	1 <sup>st</sup> fraction Ex: 9-13	2 <sup>nd</sup> fraction Ex: 11-15
D1S1656	M8			
D12S391	M8			
SE33 (ACTBP2)	M8			
FES/FPS	M8			
F13A01	M8			
F13B	M8			
LPL	M8			
Penta C	M8			
D6S1043	M8			
	M8			

2.1.2 Y-STRs

TABLE 6B

FORENSIC MODULE				
MARKER	ITEM	Total of alleles detected Ex: 13-15	1 <sup>st</sup> fraction Ex: 15	2 <sup>nd</sup> fraction Ex: 13
DYS456	M6			
DYS389 I	M6			
DYS390	M6			
DYS389 II	M6			
DYS458	M6			
DYS19	M6			
DYS385	M6			
DYS393	M6			
DYS391	M6			
DYS439 (GATA A4)	M6			
DYS635 (GATA C4)	M6			
DYS392	M6			
GATAH4	M6			
DYS437	M6			
DYS438	M6			
DYS448	M6			
DYS460 (GATA A7.1)	M6			
DYS461 (GATA A7.2)	M6			
GATAA10	M6			
DYS388	M6			
DYS576	M6			
DYS481	M6			
DYS549	M6			
DYS533	M6			
DYS570	M6			
DYS627	M6			
DYS518	M6			
DYS449	M6			
DYF387S1	M6			
	M6			
DYS456	M7			



FORENSIC MODULE				
MARKER	ITEM	Total of alleles detected Ex: 13-15	1 <sup>st</sup> fraction Ex: 15	2 <sup>nd</sup> fraction Ex: 13
DYS389 I	M7			
DYS390	M7			
DYS389 II	M7			
DYS458	M7			
DYS19	M7			
DYS385	M7			
DYS393	M7			
DYS391	M7			
DYS439 (GATA A4)	M7			
DYS635 (GATA C4)	M7			
DYS392	M7			
GATAH4	M7			
DYS437	M7			
DYS438	M7			
DYS448	M7			
DYS460 (GATA A7.1)	M7			
DYS461 (GATA A7.2)	M7			
GATAA10	M7			
DYS388	M7			
DYS576	M7			
DYS481	M7			
DYS549	M7			
DYS533	M7			
DYS570	M7			
DYS643	M7			
DYS627	M7			
DYS518	M7			
DYS449	M7			
DYF387S1	M7			
	M7			
DYS456	M8			
DYS389 I	M8			
DYS390	M8			
DYS389 II	M8			
DYS458	M8			
DYS19	M8			
DYS385	M8			
DYS393	M8			
DYS391	M8			
DYS439 (GATA A4)	M8			
DYS635 (GATA C4)	M8			
DYS392	M8			
GATAH4	M8			
DYS437	M8			
DYS438	M8			
DYS448	M8			
DYS460 (GATA A7.1)	M8			
DYS461 (GATA A7.2)	M8			

FORENSIC MODULE				
MARKER	ITEM	Total of alleles detected Ex: 13-15	1 <sup>st</sup> fraction Ex: 15	2 <sup>nd</sup> fraction Ex: 13
GATAA10	M8			
DYS388	M8			
DYS576	M8			
DYS481	M8			
DYS549	M8			
DYS533	M8			
DYS570	M8			
DYS643	M8			
DYS627	M8			
DYS518	M8			
DYS449	M8			
DYF387S1	M8			
	M8			

2.1.3 X-STRs

TABLE 6C

FORENSIC MODULE				
MARKER	ITEM	Total of alleles detected Ex: 12-15-17-20	1 <sup>st</sup> fraction Ex: 12-15	2nd fraction Ex: 17-20
HPRTB	M6			
DXS8378	M6			
DXS9898	M6			
DXS7133	M6			
GATA31E08	M6			
GATA172D05	M6			
DXS7423	M6			
DXS6809	M6			
DXS7132	M6			
DXS9902	M6			
DXS6789	M6			
DXS10103	M6			
DXS10134	M6			
DXS10074	M6			
DXS10101	M6			
DXS10135	M6			
DXS10146	M6			
DXS10079	M6			
DXS10148	M6			
	M6			
HPRTB	M7			
DXS8378	M7			
DXS9898	M7			
DXS7133	M7			
GATA31E08	M7			
GATA172D05	M7			
DXS7423	M7			
DXS6809	M7			
DXS7132	M7			

FORENSIC MODULE				
MARKER	ITEM	Total of alleles detected Ex: 12-15-17-20	1 <sup>st</sup> fraction Ex: 12-15	2nd fraction Ex: 17-20
DXS9902	M7			
DXS6789	M7			
DXS10103	M7			
DXS10134	M7			
DXS10074	M7			
DXS10101	M7			
DXS10135	M7			
DXS10146	M7			
DXS10079	M7			
DXS10148	M7			
	M7			
HPRTB	M8			
DXS8378	M8			
DXS9898	M8			
DXS7133	M8			
GATA31E08	M8			
GATA172D05	M8			
DXS7423	M8			
DXS6809	M8			
DXS7132	M8			
DXS9902	M8			
DXS6789	M8			
DXS10103	M8			
DXS10134	M8			
DXS10074	M8			
DXS10101	M8			
DXS10135	M8			
DXS10146	M8			
DXS10079	M8			
DXS10148	M8			
	M8			

**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**

FORENSIC MODULE		
		EDITED REGIONS
M6	1 <sup>st</sup> fraction	
	2 <sup>nd</sup> fraction	
M7	1 <sup>st</sup> fraction	
	2 <sup>nd</sup> fraction	
M8	1 <sup>st</sup> fraction	
	2 <sup>nd</sup> fraction	

TABLE7B

FORENSIC MODULE		
HAPLOTYPES		
M6	1 <sup>st</sup> fraction	
	2 <sup>nd</sup> fraction	
M7	1 <sup>st</sup> fraction	
	2 <sup>nd</sup> fraction	
M8	1 <sup>st</sup> fraction	
	2 <sup>nd</sup> fraction	

2.2.1 Remarks and conclusions about mitochondrial DNA results.

**3. Conclusions of the practical study**

**3.1 Forensic Module**

3.1.1 Indicate the nature of the component or components detected in the items M6, M7 and M8

	Blood	Semen	Saliva
M6	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

  

	Blood	Semen	Saliva
M7	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

  

	Blood	Semen	Saliva
M8	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

3.1.2 Indicate the minimum number of contributors detected in the items M6, M7 and M8.

	1	2	3
M6	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

  

	1	2	3
M7	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

  

	1	2	3
M8	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

**3.1.3** Could any of the donors from the reference items M1, M2, M3 have contributed to the items M6, M7 or M8?

	M1	M2	M3
M6	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

	M1	M2	M3
M7	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

	M1	M2	M3
M8	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

**3.1.4** Conclusions and remarks about items M6, M7 and M8

**4. Remarks about this exercise**

**5. Suggestions for subsequent exercises**

**6. 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?**

**Practical Forensic Module  
(Advanced 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.