



GRUPO DE HABLA ESPAÑOLA Y PORTUGUESA DE LA ISFG

GRUPO DE LÍNGUAS ESPANHOLA E PORTUGUESA DA ISFG

Instituto Nacional de Toxicología
y Ciencias ForensesSERVICIO DE GARANTÍA DE CALIDAD
DEPARTAMENTO DE MADRID

C/ José Echegaray nº 4 - 28232 Las Rozas de Madrid (Madrid)

Tf. +34 91 7688919 Fax +34 91 5648654

e-mail: intcf.eiadm@justicia.es

INTERCOMPARISON EXERCISE

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

INSTRUCTIONS EXERCISE EIADN-33 (2025)

1. SUBMITTED ITEMS AND INSTRUCTIONS FOR THEIR HANDLING

EI-ADN-33 (2025) / KINSHIP MODULE. BASIC LEVEL

- ◆ M1, M2, M3

EI-ADN-33 (2025) / FORENSIC MODULE. BASIC LEVEL

- ◆ M4, M5

EI-ADN-33 (2025) / FORENSIC MODULE

- ◆ M6, M7, M8
- ◆ M9 (for non human identification)

Reference items

Item M1 consists of a blood stain spotted of one half of a *Ahlstrom-Munksjö GenCollect* (manufactured in compliance with ISO 18385 Forensic DNA Grade Standard) half-card.

Item M2 consists of a blood stain spotted of one half of a *Ahlstrom-Munksjö GenCollect* (manufactured in compliance with ISO 18385 Forensic DNA Grade Standard) half-card.

Item M3 consists of a blood stain spotted of one half of a *Ahlstrom-Munksjö GenCollect* (manufactured in compliance with ISO 18385 Forensic DNA Grade Standard) half-card.

Forensic items

The item M4 consists of a forensic stain deposited on a substrate.

The item M5* consists of hair shafts.

Items M6, M7, M8 and M9 are unknown stains spotted onto different substrates.

Handling of items

The items consist of body fluids or hairs obtained from volunteer donors who are supposed to be healthy. Nevertheless, they should be handled as potentially hazardous material following appropriate security measures for this kind of items.

These measures include:

- *The use of gloves, mask and laboratory coat when handling and processing the items.*
- *Handling the items in a biological safety cabinet preferably.*

All precautions have been taken to ensure the homogeneity of the items, however participants must perform a representative sampling of the item as they would do in a real casework.

Great care has been taken to avoid contamination during sampling and during the preparation of the items. In the case of hair, prior to being extracted, the laboratory should eliminate a potential contamination on its surface as stated in the protocols related to the extraction of this type of item.*

The donors of the samples understand that the samples will be use anonymously and exclusively for the Intercomparison Exercise GHEP-ISFG and additionally as reference material and/or as quality control of the laboratories, being analysed by means of forensic techniques and always for the purpose of human identification, analysing noncoding regions or regions that would not provide sensitive information about the donor.

2. INSTRUCTIONS FOR COMPLETING AND SENDING THE E-FORM

IMPORTANT: The Exercise coordinator will notify by mail when the e-form is available online at <https://ghep-isfg.org/en/proficiency/>

It is compulsory to send the results through this e-form. **If there is any problem to access to the e-form, please contact us URGENTLY (intcf.eiadm@justicia.es).**

- You can access to the through the **Intercomparison Exercise** section, **2025 Form** tab.
- To login in the results form, you must enter the keys (user and password) of the person who made the registration.
- Once the e-form is completed, check the content before sending it. **If signed electronically mail it to intcf.eiadm@justicia.es or upload it to the website. If signed by hand** print all the sheets out (even if they are not filled) **and send it by postal mail.**
- **Postal mail address: Attn. Koro Fernández. Servicio de Garantía de Calidad. Instituto Nacional de Toxicología y Ciencias Forenses. C/José Echegaray, 4. 28232 Las Rozas. Madrid. España.**
- **Moreover you must upload to the website the data records of the analyses performed: electropherograms (or gels) and statistic calculations (manually made or by software generated) in order to assess the results.**
- **The results must be submitted by the closing deadline of **May 15th, 2025 22:00 GMT**; once the deadline is over it will not be possible to access to the e-form.**

TABLES 1 to 4

- In **Table 1**, report one single code by field for DNA extraction, purification/concentration and quantitation methodology.
- Please, in the case that you report 'Other', specify it in the correspondent column 00 "specify".
- In **Tables 3 & 4**, all the reference items are supposed to be analyzed with the same methodology; the same apply to both fractions of the same forensic item. Any additional comment not indicated in the former tables should be reported in section 1.5 (basic level) or in section 1.6 (advanced level)

TABLE 5

- This section must be filled in only by those laboratories taking part in the Forensic Module.
- It is compulsory to complete this section. All laboratories reporting the body fluid/s in these items, must indicate the methodology used for its identification, in order to be evaluated the nature of the fluid in the certificate. **Nevertheless, evaluation will be performed taking into account the results shown in point 3.2.1 (basic level) or in point 3.1.1 (advanced level).**

TABLES 6

- **Remember, the analysis of a minimum of 12 autosomal STR is compulsory (at least 7 STR markers must be included in the standard CODIS).**
- Before adding a new marker, please, check that it is not **yet** in the table. Blank cells will be used only for those analyzed markers **not included** in the table.
- Indicate the alleles separated by a hyphen – and leaving no blank space; in the case of homozygous genotypes, report the allele only once. Examples: X-Y X **14-15**.
- **Alleles should be reported from lower to higher order. Report microvariant alleles** indicating the number of complete repeats followed by a dot and the number of bases in the incomplete repeat. Example: 9.1-11.
- For items M1, M2 & M3 only a maximum of two alleles is allowed. The items M4, M6, M7 and M8 could be a mixture, thus more than two alleles are allowed. The alleles must be reported from lower to higher order.
- **In the case of Y-STR analysis both nomenclature proposed by NIST or by ISFG organization will be accepted.**
- **For M4, M6, M7 and M8 it's compulsory to report all the total of alleles detected (Ex: 9-11-15-17) in the shadowed column, regardless the extraction method used. The evaluation will be performed taking into account the results shown in this column.**
- In case performing differential lysis, you could also specify the alleles obtained for each fraction in the correspondent columns (first fraction/second fraction). Second fraction refers to the spermatic fraction.

TABLE 7

- **Remember you have to edit at least regions HV1 and HV2.**
- Report the initial and final positions of the edited regions (Ex. 73-340/16024-16365). However haplotypes evaluated for the certificate should be reported within positions 16024-16364 for HV1, 73-340 for HV2 and 438-574 for region HV3.
- Report the haplotypes as a list of the differences from the rCRS following medical (Ex.: A263G -315.1C A523DEL T16217C) or forensic nomenclature (Ex.: 263G 315.1C 523DEL 16217C) beginning from **the lowest nucleotide position** (that is to say, beginning in HV2 region), not leaving any space between the position and the nucleotide. Separating the differences from the rCRS **by a space without using commas.**
- **WARNING:** Pay special attention to the insertions and deletions nomenclature.
- In case that a differential lysis was performed for M4, M5, M6, M7 or M8 report the haplotype of each fraction in the corresponding row; in the case of total lysis, report the obtained haplotype in the first row.
- Remarks and conclusions about mitochondrial DNA: indicate any other result or comment you wish to mention with indication of the item to which you refer (i.e., the use of additional *primers*).

Theoretical studies

- Follow the specific instructions detailed in the corresponding sections of the form. Use the whole information provided in order to solve the theoretical studies.
- For a better management of the form, a drop-down list is provided with the name of different software, although not all of them are necessarily applicable in the resolution of each theoretical study.
- For the statistical calculations concerning autosomal STR markers, use always the allele frequencies shown **each year** in the table provided in the Appendix.
- **CAUTION: In Tables 8 & 10 and in all those sections where estimates of frequencies, LR or equivalents are required, perform the calculation with all decimal places. But in the form, report the values with four decimal places using comma as the decimal separator, round off the obtained values and show them in scientific notation (Excel format). Ex. 4,56735 should be reported as 4,5674E+00; 0,123456 should be reported as 1,2346E-01.**

ALL COMMENTS AND OBSERVATIONS MADE IN THE RESPECTIVE SECTIONS, WILL BE PUBLISHED ON THE FINAL REPORT, SO IT IS RECOMMENDED NOT TO GIVE ANY INFORMATION THAT COULD COMPROMISE THE ANONYMITY OF THE LABORATORY.

3. INSTRUCTIONS FOR SUBMITTING THE DATA RECORDS: ELECTROPHEROGRAMS (OR GELS) AND STATISTICAL CALCULATIONS

It is compulsory to submit, duly identified, the final records of the STR genetic profiles, the mtDNA sequences and the statistical calculations according to the following instructions:

3.1 Automatic platforms. STR markers (autosomal, X and Y)

Electropherograms for all the markers analyzed in every reported item must be sent. **Remember, the analysis of a minimum of 12 autosomal STR is required (at least 7 STR markers must be included in the standard CODIS).** Each electropherogram must be identified with the **item code** and the **seal number** (which will be the laboratory ID). In case you identify the electropherograms with the name of your laboratory instead of using the seal number it is understood that you waived confidentiality.

The following information must be displayed in the electropherograms:

- **Allele assignment**
- **Peak height (RFU)**
- **Allele size (pb)**

For those alleles not included in the ladder (out-of-ladder alleles), the allele size have to be shown (bp).

Electropherograms of the allelic ladders used for the analyses will also have to be submitted.

In the case of ABI sequencers, it is recommended to print out the electropherograms by using GeneMapper or Genotyper software.

Upload the electropherograms to the website (pdf file)

3.2 Manual systems. STR markers (autosomal, X and Y)

When using silver nitrate staining for fragments detection, a copy of the gel image must be submitted. Every gel copy must be identified with the **item code**, the **seal number** (laboratory ID) and the name of **every marker and allele**.

3.2 Mitochondrial DNA sequences

Aligned forward and reverse sequences with respect to the reference sequence and using the rCRS numerical notation will be sent preferably (Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, Turnbull DM, Howell N. Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat Genet. 1999 Oct;23(2):147). **Alternatively** the electropherograms obtained for each of the primers employed (Forward and Reverse) in each item should be submitted. The electropherograms must be identified with the **item code**, the **seal number** (as laboratory ID) and the **primer used**.

Colour records must be sent and upload to the website (pdf file)

3.4 Statistical calculations of the theoretical exercises

Records generated by software of all the statistical calculations required in the **Theoretical studies** must be submitted. In case of hand-made calculations formula applied must be sent. Each record must be identified with the **seal number** (as laboratory ID) and the **theoretical study** to which it corresponds.

Upload the records to the website (pdf file)

Should you have any question, please contact us: intcf.eiadn@justicia.es

- ◆ **REMEMBER THAT it is compulsory to send the form correctly filled and signed, all the original records (electropherograms and/or gels) together with the statistical data obtained by software or manually, in order to receive the certificate of participation in the Exercise.**
- ◆ **IN CASE YOU HAVE ANY DOUBT ABOUT THE ASSESSMENT, CONSULT THE PARTICIPATION RULES AT <https://ghep-isfg.org/en/proficiency/participation/>**