



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 EXERCISE

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

MASSIVE PARALLEL SEQUENCING MODULE (MPS)

INSTRUCTIONS EXERCISE EIADN- MPS 3 (2025)

1. SUBMITTED ITEMS AND INSTRUCTIONS FOR THEIR HANDLING

EIADN-MPS-3 (2025) / KINSHIP MODULE. BASIC LEVEL

- ◆ M1, M2, M3

EIADN-MPS-3 (2025) / FORENSIC MODULE. BASIC LEVEL

- ◆ M4, M5

Reference items

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

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

Item M3 consists of a blood stain spotted onto an *Ahlstrom-Muncksjö 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.

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 email when the e-form is available online at the GHEP-ISFG website: <https://ghep-isfg.org/en/proficiency/>.

Participants must send the results through that 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 eform through the **Intercomparison Exercise** section, **2025 MPS Form** tab.
- To login in the results form, you must enter the keywords (user and password) of the person who performed the registration (see letter enclosed).
- 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. Spain.**
- **You should keep MPS raw-data records** (using your platform file format: FASTQ, bam.bai, ...) in case they were necessary to perform the evaluation. In that case, a link will be provided in order to upload them. **DO NOT UPLOAD THEM TO THE EFORM.**
- **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

- In **Tables 1**, choose a code from the drop down list (see Appendix 2025).
- Please, in the case that you do not find the required option for any of the concepts, in the drop down list, specify the kit/instrument/ chip in the correspondent column 'Others' "specify".
- Use also the option 'Other' to include the versions if relevant
- **Point 1.1.5** , You have to indicate the reference sequence used in the case of STR markers (i.e GRCh37). Regarding mitochondrial DNA you have always to use the revised Cambridge Reference Sequence (rCRS)

TABLES 5

- Results from STR markers analysis (autosomal, Y-Chromosome, X-Chromosome): enter each of the alleles obtained, regardless of whether they are homozygous or heterozygous, in independent rows in the case of
- Numbers RefSNPs (rs or rsid) should be used for SNPs and/or InDels of the flanking regions according to the dbSNP database <https://www.ncbi.nlm.nih.gov/snp/>.
- When possible and if your software so permits, indicate the sequence and/or nucleotic position of the forward chain (for STR, SNP and/or InDels of the flanking regions)

TABLE 6

- **Edition of the control region is required.**
- Report the initial and final positions of the edited regions (Ex. 73-340/16024-16365).
- 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.
- Remember, you can **ONLY** report the Control Region (CR). The rest of the mtDNA genome **CAN NOT** be analyzed

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.

REMEMBER THAT it is compulsory to send the form correctly filled and signed, 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/>