

"ANALYSIS OF DNA POLYMORPHISMS IN BLOOD STAINS AND OTHER BIOLOGICAL SAMPLES"

1.-SCOPE AND ITEMS

The scope covered in the Intercomparison Exercise Program "Analysis of DNA polymorphisms in blood stains and other biological samples" includes tests for identifying fluids, genetic identification by autosomal STR markers, X-chromosome and Y-chromosome, mtDNA analysis and statistical calculations of theoretical studies.

The basic level of the intercomparison exercise program serves as proof of competence to the laboratory performing the tests. It consists of two modules:

○ **Kinship Module , which contains:**

•**Practical Study:** it entails the genetic analysis of 2-4 reference items (blood and saliva).

•**Theoretical Study:** it implies statistical calculations in a theoretical kinship study which has to be solved by the use of standardized and known formulas. Necessary data are provided (theoretical approach with silent alleles rate and mutation rate 0, assuming that the population is in Hardy-Weinberg equilibrium and that no correction is made due to population substructure, allele frequencies to use in the calculations, parameters to consider...) to calculate genotype frequencies, Paternity Indices (PI) or Likelihood ratios (LR).

○ **Forensic module , which contains:**

•**Practical Study:** it involves analyzing genetically 2 unknown forensic items: one consisting of any human biological fluid or of a mixture of body fluids with a maximum of two contributors, in a volumetric proportion not more than 1:2 (v/v) in case they are different fluids and in 1:3 if it is the same fluid. Body fluid identification is also requested. The other item consists of a hair or hairshaft in which analysis of mitochondrial DNA is requested.

•**Theoretical Study:** it implies statistical calculations in a case of forensic interest which has to be solved by the use of standardized and known formulas. Necessary data are provided (theoretical approach with silent alleles rate and mutation rate 0, assuming that the population is in Hardy-Weinberg equilibrium and that no correction is made due to population substructure, allele frequencies to use in the calculations, parameters to consider...) to calculate statistical parameters of forensic interest such as genotype frequencies, match probabilities, *Likelihood ratios* (LR).

The advanced level constitutes a tool for introducing new items both practical or analytical and for implementing new techniques. It contains two modules:

- **Kinship module**
 - **Practical study:** No items are submitted
 - **Kinship paper challenge:** participants are asked to solve a proposed paper challenge.
- **Forensic Module**
 - **Practical study:** the genetic analysis and body fluid identification of forensic items are requested. These may contain fluids of human and/or non-human origin.
 - **Forensic challenge:** participants are asked to solve a proposed paper challenge.

2. - DEFINITIONS

Sample: biological fluids or hair taken from a donor.

Item: test sample used in a proficiency test. They consist of biological fluids put on a substrate, clean or contaminated hair with any biological fluid and data.

3. -TIMETABLE

The Exercise is held annually, following as much as possible the schedule below:

- October-November: Preparation of items
- November-December: Registration
- End of January or early February: Deadline for payment of participation fees
- 1-20 February: Delivery of items
- 1 month before the end of the deadline for sending results : the e-form is enabled on the website
- 1 to 15 May: Deadline for submission of results
- In May, after the deadline for submission of results: results summary is sent
- In September, the Meeting organized by laboratories members of the GHEP-ISFG group is held.
- After the Meeting: Final Report
- In October: certificates of participation and evaluation of results.

The schedule depends on the dates when the Meeting is held, and therefore it may be subject to variations that will be conveniently communicated to participants.

4.-PARTICIPATING LABORATORIES AND REGISTRATION

- Registration is free and open to all interested laboratories, whose staff can be members of the Spanish and Portuguese-speaking working group of ISFG (GHEP-ISFG) or not, both public or private laboratories that carry out biological research of paternity and/or analysis of DNA polymorphisms for use in the forensic field.
- Annually, the registration is done through an electronic form available on the website of the GHEP-ISFG group during an established schedule. Previously, the

coordinator informs participants from previous years and the secretary informs the members of the GHEP–ISFG group of its availability. A few days before the closure of registration all laboratories receive a reminder.

- Enrollment in the program implicitly represents the recognition and acceptance of the conditions inherent in the organization and the requirements established by the department of Madrid of the National Institute of Toxicology and Forensic Sciences (INTCFM) and the GHEP-ISFG. The INTCFM/GHEP-ISFG reserves the right not to accept applications from laboratories that in previous years have failed to fulfill the contract.
- Enrollment in the Basic Kinship module level is compulsory.
- **Registrations outside the deadline will not be accepted. In order to receive the items, the laboratory must not only be registered but also have done the payment of the participation fee, within the schedule.**
- **A seal number will be assigned to the laboratory with which it will be identified throughout the Exercise.**
- Each module of each level of the program has a fee with which the GHEP-ISFG group manages to cover the costs of organization. The kinship module is the minimum that must be paid since such registration is compulsory. Fees are listed on the website of the GHEP-ISFG group.

4.1- Compromises to be met by participants

- The participant laboratory assumes that they will do an anonymous and exclusive use of these items for the Intercomparison Exercise INTCFM/GHEP-ISFG or as a reference material for assessing the quality of their laboratory, by signing the written submission results form.
- The participants agree to perform laboratory tests in their facilities and with their personnel using similar procedures as those followed in real casework. They also compromise to work under appropriate hygiene and safety measures.
- The participating laboratory is committed to provide the means to prevent collusion.
- Participation with a minimum of 12 autosomal STRs (at least 7 standard CODIS STRs) and the edition of HVI and HVII mitochondrial DNA regions are mandatory. Furthermore, the study will be conducted with the markers and the laboratory methods currently used in routine or that are being developed.
- Theoretical calculations have to be always performed according to the allele frequencies provided.

- **The participating laboratory must send the original results (electropherograms or copies of gels) together with copies of the records from the statistical calculations.**
- The laboratory shall send the results in due time.
- **The participating laboratory has to inform the provider about the correct receipt of the items.**

5. - INSTRUCTIONS AND DELIVERY OF ITEMS

The supplier will inform the participating laboratories by email the date and method of shipment, and will send the necessary instructions for the analysis of the items. The documentation will include the deadline (date and time) for sending results.

The means of transport chosen to deliver the items will ensure the traceability of the delivery. To do so, the delivery is carried out by a transport agency or other (Embassies by diplomatic bag or another choice of provider) in order to guarantee the reception of the items in the shortest time possible and with the maximum safety.

Moreover, if a problem arises, the participant laboratory will have 10 days to request another delivery, but this will not entail a change in the established deadline for submission of results.

6. - THE RESULTS FORMS AND HOW TO SEND THEM

The results have to be sent through the electronic form of the GHEP-ISFG group website. The laboratory will receive by mail information about its availability, approximately, a month before the deadline for sending results. The laboratories, will also receive a reminder a week before the end of that term. The form will be disabled immediately after the date and time scheduled for the deadline, the results being not admitted if received subsequently.

In the forms, the sections of the exercises that will be evaluated and certified will be identified.

It is compulsory to send a signed and complete copy of the form with all paper sheets. By signing this form, the laboratory agrees to make an exclusive use of the items for the exercise and as reference material, so it is essential to send this document to the coordinator in order to receive the certificate.

Digital and printed versions should have the same content. **In addition, it is requested the remittance of electropherograms and/or copies of gels together with records for statistical calculations for evaluating the results. These records can be printed or sent by email. Laboratories that do not send any of these records will not be evaluated.**

It is the responsibility of the laboratory to identify these records as described in the instructions, eliminating any data which could allow the identification of the laboratory and compromise anonymity.

It will be also reminded in the instructions to the participants that all comments made in the spaces provided will be published in the final report. So it is recommended not to give any information that might compromise the laboratory's anonymity.

Corrections of data and/or results will not be allowed after the deadline.

For any query related to the documentation sent, participants should contact the addresses and telephone numbers which are indicated in the instructions.

7. - STATISTICAL DESIGN AND ESTABLISHMENT OF ASSIGNED VALUES

The intercomparison program will be developed following the requirements of the UNE-EN ISO/IEC 17043:2010.

BASIC LEVEL

Definitions

Assigned value: Evaluation will be carried out with regard to assigned values. These values can be established by consensus from participants or can be known values.

Consensus value: to agree on a result there must be a minimum participation of 10 laboratories and concordance of results in at least 70% of the participants.

Known value: value obtained by a selected and accredited laboratory, whose scope covers the testing procedures used to establish the known values or by means of the use of standardized statistics formula.

7.1 - Practical Studies

7.1.1- Criteria for value assignment of items from kinship module

The assigned values are established by consensus . These criteria apply to the genetic analysis of autosomal STR markers, Y-chromosome STR markers, X-chromosome STR markers and haplotype analysis of mitochondrial DNA.

If a consensus is not reached for a marker no assigned values will be set.

7.1.2 -Criteria for value assignment of items from the forensic module.

7.1.2.1 - Assigned values from known values will be applied in the following cases:

- **Identification of the type of fluid or fluids of the item:** it is a known value.

• **Genetic analysis:** A selected and accredited laboratory analyzes independently two DNA extracts from the same contributor. Each extract is amplified with, at least, two different kits from two different commercial companies, which together, they cover the Amelogenin, autosomal STR markers and Y-Chromosome STR markers **that are outlined below**

Autosomal STR markers + Amelogenin :

D3S1358, vWA, D16S539, CSF1PO, TPOX, D8S1179, D21S11, D18S51, D2S441, D19S433, TH01, FGA, D22S1045, D5S818, D13S317, D7S820, SE33, D10S1248, D1S1656, D12S391, D2S1338, Penta E and Penta D.

Y-Chromosome STR markers:

DYS576, DYS 389I, DYS448, DYS389II, DYS19, DYS391, DYS481, DYS549, DYS533, DYS438, DYS437, DYS570, DYS635, DYS390, DYS439, DYS392, DYS643, DYS393, DYS458, DYS385, DYS456, GATA -H4.

7.1.2.2 - Assigned values by consensus will be applied in the following cases and only in items with one component (unique source):

X-Chromosome STR markers and mitochondrial DNA

7.2 - Theoretical Studies

In order to achieve uniformity in the results and make evaluation possible, all theoretical calculations have to be done with the frequencies provided.

The assigned value is a known value established by the use of standardized formulas.

ADVANCED LEVEL

Definitions

Assigned value: Evaluation will be carried out with regard to assigned values. These values can be established by consensus from participants or can be known values.

Consensus values: to agree on a result there must be a minimum participation of 5 laboratories and concordance of results in at least 70% of the participants.

Known value: value obtained by a selected and accredited laboratory or through the use of standardized formulas.

7.3 -Practical studies

7.3.1- Criteria for value assignment of items from the forensic module

7.3.1.1- Assigned values from known values will be applied in the following cases:

- **Identification of the type of fluid or fluids of the item:** It is a known value.

7.3.1.2- Assigned values from known values or established by consensus will be applied in the following cases:

- **Genetic analysis:** Amelogenin, autosomal and Y-Chromosome STRs indicated below. Assigned values are established by consensus **only if** there are no known values established, otherwise known values will always prevail. In case of evaluations based on known values, the following markers are evaluated:

Autosomal STR markers + Amelogenin :

D3S1358, vWA, D16S539, CSF1PO, TPOX, D8S1179, D21S11, D18S51, D2S441, D19S433, TH01, FGA, D22S1045, D5S818, D13S317, D7S820, SE33, D10S1248, D1S1656, D12S391, D2S1338, Penta E y Penta D.

Y-Chromosome STR markers:

DYS576, DYS 389I, DYS448, DYS389II, DYS19, DYS391, DYS481, DYS549, DYS533, DYS438, DYS437, DYS570, DYS635, DYS390, DYS439, DYS392, DYS643, DYS393, DYS458, DYS385, DYS456, GATA-H4.

7.3.1.3- Assigned values by consensus will be applied in the following cases and only in items with one component (unique source):

X-Chromosome STR markers and mtDNA

7.4- Challenges

All the results sent by the participants will appear on the final report together with the known value provided by the advisor but **no certificate will be issued for the challenges.**

8. -REPORTS OF RESULTS

It is planned to issue a results summary and a final report.

- The results summary is a preview of results. It includes their distribution among the laboratories. This summary is electronically sent to all participants and it will also be available on the website of the GHEP-ISFG group after the deadline for submission of results.
- The **final report** will be available after the annual meeting (GHEP-ISFG Meeting). In

this report all information submitted by each of the laboratories is exposed, including methodologies and results, assigned values, considerations and recommendations which may be deemed appropriate after the results obtained.

Participants will be warned that the data and results in both summary and final report cannot be reproduced in other means different from that detailed, without the expressed permission of the Coordinator.

If any mistakes are detected in any of the reports, a new one will be issued with the corrections and justification of corrections. It will be clearly identified as a new report which will substitute the previous one.

9. -WORKING GROUP MEETING

The GHEP-ISFG group organizes an annual meeting (Working group Meeting GHEP-ISFG) for discussion of the results of the corresponding year. In this conference a discussion on the values obtained is set providing a forum for discussion to help laboratories to self-assess their results.

During the Meeting the Executive Committee of the GHEP-ISFG meet the Coordinator of the Exercise in order to review the Program, advise him/her on possible guidelines to follow for the next edition of the Exercise and establish new improvement actions

Subsequently on the website of the GHEP-ISFG group, the presentations, summaries of these discussions and the decisions taken are published, together with the final report.

10. -EVALUATION OF RESULTS

The assessment of the results will be carried out with regard to the assigned values published on the final report. The values reported by the laboratory in the form and those reported in the original records must coincide and, in case they do not, discrepancies will be studied in order to carry out the evaluation.

Evaluation of results will be carried out taking into account the following points:

- **Body fluid identification:** The establishment of the nature of the fluid or fluids has to be supported by a technique, otherwise the answer will not be evaluated.
- **STR genetic analysis:** In the case of the expression of results obtained in the analysis of Y chromosome STR markers, both nomenclature proposed by the ISFG organization or by NIST will be accepted. In case of genetic analysis of

mixtures, X-Chromosome STR profiles will not be evaluated for the certificate, although general results obtained by the laboratories will be informed in the final report. In the case of reporting results for autosomal STR markers and Y-Chromosome STR markers different from those referred above (point 7.1.2.1), these will appear on the report but they will not be evaluated for the certificate. In the case of value assignment from the known value, if there were any discrepancies in the analysis of any of the markers carried out by the reference laboratory with different, the assigned value would not be given for that marker.

- **Mitochondrial DNA:** Laboratories may submit results for all regions of mitochondrial DNA that are edited, however the haplotypes evaluated on the certificate will be the following positions: HV1 region between positions 16024-16365, HV2 region between 73-340. Regarding the HV3 region, the evaluation will be done in the editing range between positions 438-574, sequences which do not include this range will be not evaluated. The length variants present in the homopolymeric tracts will not be assessed. This includes insertions or deletions in the length variants that may appear in the positions: 16193, 309, 455, 463 and 573. It is compulsory the analysis of the HV1 and Hv2 regions, the analysis of only of one of these regions will not be taken into account for evaluation. Each of the mtDNA regions analyzed will be evaluated separately. . In case of genetic analysis of mixtures, mitochondrial DNA haplotypes will not be evaluated for the certificate.
- **In the practical kinship study,** the reference samples will be assessed individually according to the consensus reached in each one.

For the evaluation of the results, the Coordinator may receive technical assistance from an advisory group, ensuring at all times the confidentiality of the results

The nomenclature used to express the evaluation will be as follows

- **For the evaluation of the practical exercises**

- **C:** Correct, it matches with the assigned value, which may be a known value or obtained by consensus.
- **F/C:** It is correct, it matches with the assigned value but it differs from the format specified in the instructions
- **D:** discordant results, which include the rest of discrepancies due to errors in typing, allelic losses or gains, change of sample, etc.

- **N:** Discrepancies due to the use of a nomenclature different from the one internationally recognized.
- **T:** transcription errors in completing the form. They considered as such any discrepancy between what the laboratory has reported on the form and the original primary records submitted on time, provided that the result of the primary record matches the assigned value.
- **NA:** Not analyzed.
- **SR:** Without results. Analyses are performed but no results are obtained. They are considered as discrepancy.

• **For the evaluation of the theoretical exercises (only basic level)**

- **C:** Correct, result which match with the assigned value. The assigned value is the theoretical known value provided by the consultant and obtain by means of standardized formulas.
- **A:** Acceptable results within the range : assigned value \pm 5%.
- **F/C:** It is correct, it matches with the assigned value but it differs from the format specified in the instructions.
- **F/A:** It is acceptable but it differs from the format specified in the instructions.
- **D:** discordant results, they are nor correct neither acceptable.
- **T:** transcription errors in completing the form.
- **NA:** Not analyzed.

When it is not possible to establish an assigned value for a parameter, it will be express as (SA).

11. - ISSUANCE OF CERTIFICATES OF PARTICIPATION AND EVALUATION OF RESULTS

After evaluating the results, the certificates of participation and assessment of results to the participating laboratories are issued. They are signed by the Coordinator with the agreement of the INTCFM Director and the President of the GHEP-ISFG group.

In each certificate it is indicated where to consult the assigned values from which evaluation has been made.

For each module a certificate of participation and evaluation of results is issued. For those laboratories that having taken part in the Exercise do not submit original data (electropherograms or gels) or records from statistical calculations, the certificate of participation will not be issued.

In all the certificates, laboratories are identified by their seal code of participation and in an enclosed letter, signed by the Coordinator, the correspondence between the seal

code and the number of the laboratory is displayed, specifying the certificates that are being sent. Moreover, in that letter the participants are informed that in case of any complaint about the evaluation of performance, there is a Complaint form at their disposal.

The characterization of the forensic sample is evaluated based on the number of detected contributors, the contribution of any of the reference samples and on the identification of the nature of the fluid or fluids that compose it.

Markers, analyzed by the laboratory, for which an assigned value has been established, are included in the certificate. Moreover results from the characterization of the forensic item (number of contributors detected, possible contribution of one of the reference items and identification of the nature of the fluid or fluids) and the evaluation of the theoretical exercises are shown.

When a laboratory does not inform a particular statistical value or marker, those parameters will be denoted in the certificate as not analyzed (NA).

If any mistakes are detected in any of the certificates, a new one will be issued with the corrections, clearly identifying that it will substitute the previous one.

12. -CONFIDENTIALITY

All information received by the INTCFM about participants will be treated with complete confidentiality, including the evaluation obtained and will not be disclosed to a third party unless explicit acceptance of the laboratory for a particular purpose, known *a priori*.

In the report, the identification of the participants will remain anonymous through the seal code. This code varies annually and it is provided by the supplier, ensuring in this way the traceability and confidentiality of the results.

In the instructions, participants are asked to mark all submitted documents related to the Exercise (result forms, electropherograms, claims, etc ...) with the annual seal code. It is the responsibility of the laboratory to follow these instructions to ensure the confidentiality of the communication.

13. -QUALITY ASSURANCE

The INTCFM agrees to follow all the requirements of the UNE-EN ISO/IEC 17043:2010, which are included along with those reported in the UNE-EN ISO/IEC 17025:2005, in the Quality System of the INTCF of Madrid.

The INTCFM will carry out the necessary measures to ensure stability and homogeneity of the items among the participants.

14. - COMPLAINTS

On the GHEP-ISFG website, a Complaint form is available which after completion will be forwarded to the program coordinator. This form will be able to attend all types of complaints and reclaims concerning the exercise, including those against performance evaluation. In case a complaint leads to a non-conformance and to a corrective or a preventive action, they will be managed as set out in the Quality Manual of the INTCFM. All complaints will be answered directly to the participant. In both cases the participant will receive an answer about the decision made or about any related explanation by email.