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Research paper

GHEP-ISFG collaborative exercise on mixture profiles (GHEP-MIX06). Reporting conclusions: Results and evaluation

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ABSTRACT

One of the main goals of the Spanish and Portuguese-Speaking Group of the International Society for Forensic Genetics (GHEP-ISFG) is to promote and contribute to the development and dissemination of scientific knowledge in the field of forensic genetics. Due to this fact, GHEP-ISFG holds different working commissions that are set up to develop activities in scientific aspects of general interest. One of them, the Mixture Commission of GHEP-ISFG, has organized annually, since 2009, a collaborative exercise on analysis and interpretation of autosomal short tandem repeat (STR) mixture profiles. Until now, six exercises have been organized. At the present edition (GHEP-MIX06), with 25 participant laboratories, the exercise main aim was to assess mixture profiles results by issuing a report, from the proposal of a complex mock case.

One of the conclusions obtained from this exercise is the increasing tendency of participating laboratories to validate DNA mixture profiles analysis following international recommendations. However, the results have shown some differences among them regarding the edition and also the interpretation of mixture profiles. Besides, although the last revision of ISO/IEC 17025:2017 gives indications of how results should be reported, not all laboratories strictly follow their recommendations.

Regarding the statistical aspect, all those laboratories that have performed statistical evaluation of the data have employed the likelihood ratio (LR) as a parameter to evaluate the statistical compatibility. However, LR

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values obtained show a wide range of variation. This fact could not be attributed to the software employed, since the vast majority of laboratories that performed LR calculation employed the same software (*LRmixStudio*). Thus, the final allelic composition of the edited mixture profile and the parameters employed in the software could explain this data dispersion. This highlights the need, for each laboratory, to define through internal validations its criteria for editing and interpreting mixtures, and to continuous train in software handling.

1. Introduction

At present, the typing of STRs markers is the gold standard in the field of Forensic Genetics. But the interpretation and evaluation of mixture profiles are undoubtedly one of the main difficulties forensic laboratories find in their daily work. Numerous scientific working groups have published recommendations and guidelines to address the analysis and assessment of these types of profiles [1–10]. However, despite them, the interpretative difficulty and the lack of a unique criterion are important challenges for the laboratories to cope with.

With respect to the statistical evaluation of mixture profiles, the use of LR is recommended [2] and widely accepted. The complexity of some of these profiles sometimes makes this evaluation unfeasible to be done manually. Thus, different computer programs have been developed over the past years (e.g., *DNAMIX* [11], *Grape* [12], *DNAmixture* [13,14], *Forensim* [15]), and more recently, some others that allow introducing additional variables to deal with complex mixed profiles produced by sensitivity of the current DNA technology, with the presence of drop-out, drop-in or other artifacts (e.g., *LRmixStudio* [16,17], *EuroForMix* [18–20]), as international recommendations indicate [10,21]. But, the use of any of these software must be validated according to international recommendations [22], before its application in the daily routine.

In addition, results expression is another important point when dealing with mixture profiles, whose final representation is report issuance. Some authors have shown the bias in forensic DNA mixture interpretation as a result of the absence or presence of context information about the criminal case [23]. But even with the same information, there are sometimes great differences as indictment or exoneration of a suspect in the face of the same mixture [23,24]. For this reason, a few guidelines have to be followed [25], and in that sense, European Network of Forensic Science Institutes (ENFSI) has made a significant effort [26].

Therefore, a new edition (GHEP-MIX06) was proposed from Mixture Commission of the GHEP-ISFG in 2015, which was being carried out since 2009 [27]. In this new edition (GHEP-MIX06), in addition to evaluating the editing and interpretation of mixed profiles, as in previous editions [27], the expression of results will also be evaluated through the issuance of a report by the participant laboratories. This paper shows the results and conclusions that have been generated over this last edition of the exercise.

2. Materials and methods

2.1. Participants

As previous exercises [27], this collaborative exercise was open to all laboratories with GHEP-ISFG members. A total of 25 laboratories from 8 different countries have been involved: 12 from Spain, 3 from Portugal, 3 from Argentina, 2 from Chile, 2 from Italy, 1 from Colombia, 1 from the Dominican Republic and 1 from the Czech Republic. Most of the participant laboratories belonged to public institutions (10 Justice Administration Labs, 9 Health and University Labs and 4 Police Labs), and only 2 ones to private companies.

Although most of the laboratories developed their work in the criminal field (both forensic and paternity cases), there was one laboratory that exclusively performed paternity testing.

2.2. Exercise scheme

This exercise was organized and coordinated by the Mixture Commission of the GHEP-ISFG. The participants received two genetic mixture profiles in PDF format, pre-analyzed by organizer with internal parameters (50 RFUs of Analytic Threshold −AT−). With these mixture profiles and supplementary mock case information, participant laboratories were asked to write a complete report as their institution usually emits.

Additionally, a questionnaire was included with some issues about the characteristics of the laboratory, as well as the technical criteria used in the interpretation of profiles and statistical estimation.

2.2.1. Questionnaire design

As on previous editions [27], along with the genetic mixture profiles, a questionnaire was also provided to the laboratories with the main goal of collecting information regarding general aspects related to the characteristics of the laboratory, methodological issues that deal with the interpretation of the mixture profile, and also aspects related to the statistical treatment (see Supplementary material A3). Regarding the results obtained for the different samples, the questionnaire also included tables for reporting their profiles (see Supplementary material A3, to see the results of the participants).

2.2.2. Samples

A total of 2 samples were analyzed in this edition. Mixtures were prepared artificially using DNA extracted from buccal epithelium samples provided by anonymous donors. DNA extracts had been previously quantified in duplicate (*Quantifiler® Duo*), in order to optimize as much as possible the correct ratio between the components of the mixture. The proportion and the number of contributors of the mixture samples were variable. Once the mixture sample was set up in the work proportion, the DNA extract was quantified again for estimating the optimal DNA input to be employed in the amplification reaction to produce the required mixture.

These extracts (1 μL of each of them) were analyzed with different commercial kits: *AmpFlSTR® NGM™*: M1 for autosomal markers (1:3:7, male-female-male) (see profile in Supplementary material A1); and *AmpFlSTR® Yfiler™*: M1 for Y-chromosome markers (1:3, male-male) (see profile in Supplementary material A2). The amplification conditions for both kits were those provided by the manufacturer. And the CE in ABI 310 was carried out with the standard setting validated in the organizer laboratory (5 s/15 kV). Then, PDFs were generated from the appropriate electropherograms (EPGs), and they were modified using Adobe® Photoshop® CS2 version 9.0 for the requirements of the exercise.

Participants were provided with the thresholds values used/employed: analytical threshold of 50 RFUs, stochastic threshold of 150 RFUs, and stutter threshold for each of the markers/kits according to the manufacturer's specifications.

2.2.3. Mock case

The main point of this exercise was to assess how each of the participating laboratories would report those results on mixtures profiles (from autosomal and Y-chromosome markers).

For this purpose, a mock case was presented (see Supplementary material A4). Besides, the results obtained by a hypothetical laboratory (including the mixtures profiles indicated in the above section) were

given, considering that they were a final point and could not be extended with further analysis. From these results, the participants had to issue a complete report, including the different sections of their institution report (i.e., “background”, “received samples”, “analyzed samples”, “date of analysis”, “results”, “hypotheses raised”, “conclusions”, “notes”, “bibliography”, …).

Participants had to consider the exercise as a real case and handled it according to their laboratories' procedures. If they considered appropriate, LR calculations were performed using hypotheses that were formulated by the participants themselves. LRs had to be computed for each locus as well as for the overall profile, using Spanish population frequencies [28,29] and without correction for subpopulation structure.

2.2.4. Assessment reports

From each participating laboratory issued report, a detailed evaluation was made (see Supplementary material A5). A detailed quantitative assessment was carried out for the number of pages issued in the different sections of the report: description of samples, analyses, glossary, results, interpretations, conclusions or bibliography.

On the other hand, it was assessed how many laboratories recorded the different components within each section. In addition, in those reports including statistical evaluation of the results, we recorded how many laboratories formulated each type of hypotheses pair, as well as the value of LR obtained. Finally, it was assessed the conclusions obtained by each of the participating laboratories and how they were expressed.

3. Results and discussion

3.1. Participants characterization

Extensive details about the questionnaire answers are given in Tables S1–S3, in the Supplementary data of Appendix A3.

Firstly, it can be highlighted that the 96% of the participating laboratories developed their activity both in the criminal and in the civil fields (paternity testing) (Table S1, Appendix A3). In addition, more than half of the laboratories registered their genetic profiles to a database (32% to the National database and 24% to the National and own database).

Regarding mixture DNA interpretation, 80% of the laboratories indicated they performed the interpretation of this type of profile in their routine casework results. A minority of them claimed to carry out this interpretation exclusively in cases where reference samples were available (12%). On the other hand, many laboratories (48%) replied that this kind of profile was registered on both, reports emitted to the court and registered to a national DNA database.

The majority of participants (96%) had performed the allelic assignments of the mixture components manually as well as using some software. This information seems more consistent with the real daily practice of a forensic laboratory.

Finally, the questionnaire of the exercise requested the participants if the criteria used to carry out the interpretation of mixture profiles had been validated according to international requirements [30,31]. A total of 44% of participating laboratories answered positively, 20% answered negatively, and the rest, 36%, was validating their methods for mixtures profile interpretation.

3.2. Profile characterization

3.2.1. Parameters used for the analysis of profiles in the routine casework

Table S2 (Appendix A3) shows detailed information about this part of the questionnaire.

The questionnaire included one second block of questions related to technical and methodological aspects used to edit mixture profiles on the participating laboratories work routine (i.e., threshold values, software employed and criteria to characterize a profile as a mixture). Also, in this block of questions, participants were inquired about the main difficulties they usually faced to carry out the interpretation of this type of mixture profiles.

Firstly, regarding the software used for editing the EPGs of the samples, most of the participating laboratories (92%) employed the *GeneMapper*™ (Applied Biosystems) software (different versions). However, other programs were also used (*Genotyper*® or *Grape* software).

Concerning the criteria used to define profile as a mixture, there was great agreement among the participating laboratories. Most of the participants (52%) did not employ a single criterion, but a combination of several to recognize a profile as a mixture. They considered that two

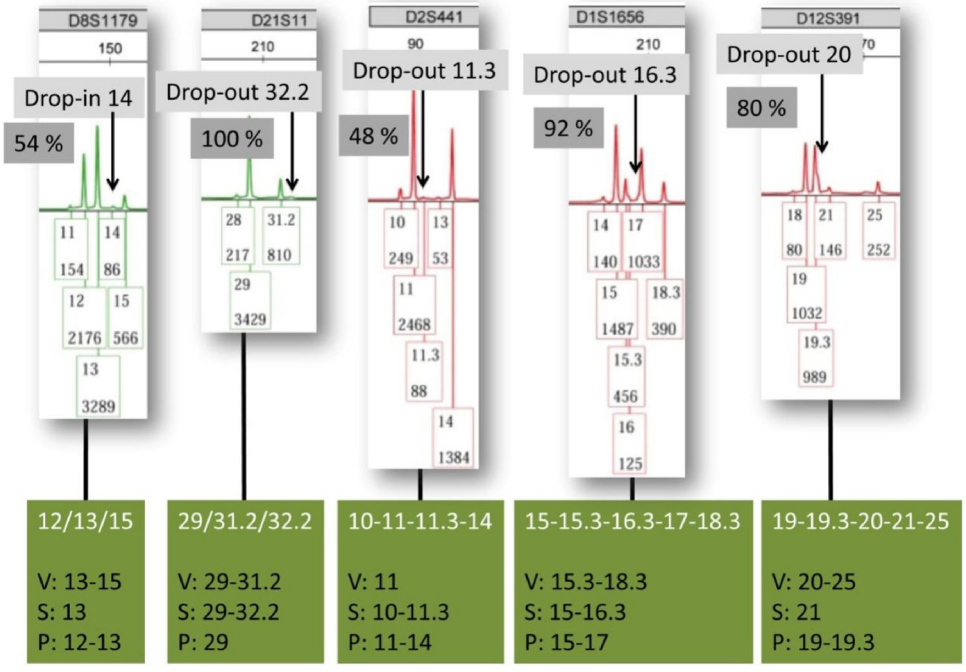

Fig. 1. Principal problematic markers in the Autosomal Mixture Profile. Legend: V (Victim), S (Suspect), P (Regular partner).

conditions should be met to characterize a profile as a mixture: the presence of at least two genetic markers with at least 3 alleles each and the existence of allele imbalance. On the other hand, the laboratories were asked about the relative fluorescence units (RFU) value employed for the analytical threshold [6,32]. In this case, it apparently seems that there was enough consensus among laboratories since most of the participants (76%) established that value at 50 RFUs.

Another challenge on mixture profile interpretation was *stutter* positions, because of difficulties to distinguish between a real allele and an artifact (*stutter*) [2,27]. Participants were questioned about when a possible peak detected in a *stutter* position was assigned as a true allele, regardless that these were in position n-4 (n-3 in the case of the D22S1045 marker). The variability of the responses from the participants showed the lack of a single criterion. Practically half of the laboratories (40%) indicated that "the assignment was variable depending on the STR marker", and the other half (40%), "the assignment has been variable depending on which STR marker is taken into account, and > 50 RFUs".

Finally, when participants were asked about the main obstacles they encounter in interpreting mixed profiles, the answers denoted that the main problems were the lack of a single criterion within the same laboratory, as well as the lack of the necessary and suitable training, and other factors, such as the nature of the sample or combination of different factors.

3.2.2. Statistical aspects

As in previous editions [27], the GHEP-MIX06 exercises also included a section consisting of carrying out a statistical assessment of the compatibility between known genetic profiles (reference samples from the victim and/or suspect/s, known contributors) and the mixture profiles corresponding to samples sent in the context of the mock cases (Table S3, Appendix A3). In this sense, following the recommendations of the ISFG [I2], a large majority of participants (76%) employed the likelihood ratio statistic (LR) as the most appropriate approach for statistical evaluation for the autosomal mixture profile. However, regarding Y-chromosome mixture profile, the majority of the participants (52%) would not make a statistical evaluation of this profile

Regarding the method employed for statistic calculation by the laboratory, the majority of participants (88%) would exclusively carry out LR calculations using software (e.g., *LRmixStudio*, *EuroForMix*, *DNAMIX*). Among the software used, *LRmixStudio* stands out which is utilized by 64% of the participants.

3.2.3. Remarkable results/discrepancies

In relation to autosomal mixture profile, the most problematic markers are (see Fig. 1): D8S1179, where 54% of laboratories report the allele 14, which is, in fact, a *drop-in*; D21S11, where 100% of laboratories do not report the allele 32.2 (*drop-out*); D2S441, where 48% of laboratories do not report the allele 11.3 (*drop-out*); D1S1656, where 92% of laboratories do not report the allele 16.3 (*drop-out*); and D12S391 marker, where 80% of laboratories do not report the allele 20 (*drop-out*). In some cases, where the *drop-outs* are clear, as they do not appear in the EPG (D1S1656), it is possible that some laboratories (the remaining 8%) made a biased interpretation of the mixture considering the reference profiles provided. On the other hand, with respect to D12S391 marker, although the allele 20 did not appear in the EPG, it is clearly guessed as a hump of the allele 19.3, and for that reason, it is possible that 20% of the laboratories did report their presence.

With respect to Y-chromosome mixture profile, there was a consensus among laboratories when performing the allelic assignment. The major discrepancies lie in the nomenclature of alleles. Thus, some laboratories highlight somehow those alleles that are found to a greater or lesser proportion (with some specific notation). In the case of the GATAH4.1 (H4) marker, the majority of labs prefer the nomenclature of the long fragment as GATAH4, although two labs (8%) report both nomenclatures, and only 28% (7 laboratories) prefer GATAH4.1 nomenclature [33,34].

3.3. Assessment reports

A first evaluation was made based on the number of issued pages in the reports sent by the participants. Only 3 laboratories included annexes as such, with a range of total page numbers (report plus annexes) between 2 and 10 (average of 7.33 pages). And for the rest (22 labs) issued reports have a page number range from 1 to 11 pages, which means an average of 6.16 pages.

Evaluating the sections included in the report, the average number of pages in each section is included in the following graph (Fig. 2). The most extensive section is "Results" with an average of 2.14 pages, followed by "Conclusions" with an average of 0.95 pages. The following in extension would be the "Performed Analyses" (0.77 pages) and the "Description of the Samples" (0.71 pages).

3.3.1. Sections of reports

Extensive details on the assessment of each section of reports are included in the Supplementary data of Appendix A5. Below some of the main aspects will be highlighted. The analysis was focused on technical requirements of ISO/IEC 17025:2017 (point 7.8) [30].

Header

Almost all submitted reports (96%) included any kind of headline on the first page. More details of the parts of the header are included in Appendix A5 (page 2). In the rest of the pages, only 80% of the

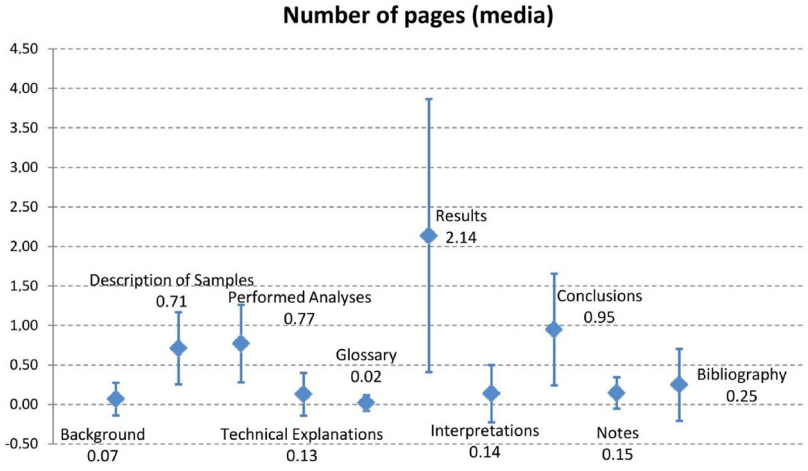

Fig. 2. Number of pages Assessment for each Sections' Reports.

laboratories included some type of heading. And surprisingly, only 60% included at least the identification that has been assigned by the laboratory to the case. This fact could be somewhat equivocal. It should be recommended that all pages of the reports be identified at least with the reference assigned by the submitting body and/or by the laboratory issuing the report. In the best-case scenarios, both references must appear on all pages of the report to ensure the authenticity and integrity of the text issued by the laboratory. In this regard, the previous version of ISO/IEC 17025:2017 [30] (ISO/IEC 17025:2005 at section 5.10.2, paragraph c, Note 1) also includes the convenience that "Hard copies of test reports and calibration certificates should also include the page number and total number of pages".

Preamble

92% of the laboratories included this section. All of these laboratories at least included the reception date of the case, the court that interests and the references assigned by this court in this section. And only 72% of the laboratories headed the report with a title (e.g., "Biology-DNA Report" or similar), as ISO/IEC 17025:2017 states (section 7.8.2.1, paragraph a) [30].

Background

20% of the laboratories included information related to the background of the investigated case in the report. In addition, only 2 laboratories (8%) recorded some information about submitted evidence chain of custody in this section.

Received Samples/Evidence

All the participating laboratories entered this section in their reports. Most (92%) described and identified the submitted samples, as it was recommended by ISO/IEC 17025:2017 (section 7.8.2.1, paragraph g) [30]. It is surprising that just over half (52%) separated the shipments or specified the organisms that made each shipment.

Analyzed samples

Not all laboratories recorded this section; only 52% of them jointly indicated "Received Samples/Evidence" and "Analyzed Samples" sections. In addition, it should be emphasized that in the body of the submitted reports, 44% of the laboratories jointly specified both the code and the description of the sample to which they refer, and only 8% (2 laboratories) referred to the samples through the code that has been assigned to them. For a traceability issue, the body of the report should at least indicate the coding of the samples analyzed, the same as indicated/assigned in the sections of "Received Samples/Pieces of evidence" and/or "Analyzed Samples". But in no case only the description of the samples, as it may be misleading. In this way, it would also be possible to maintain a continuous and adequate reading of the report (without the need to constantly resort to previous sections).

Requested analyses

Only 80% of the laboratories registered a section with the requested analyses. If this section does not appear in the reports, it would not represent a major problem whether the laboratories register the following section, "Performed analyses".

Performed analyses

8% of the laboratories (2 labs) did not record the performed analyses, as ISO/IEC 17025:2017 clearly reflects (section 7.8.2.1, paragraph f) [30]. Obviously, another fact would be to evaluate which methods were registered (see page 6 of Supplementary data of Appendix A5), since not all laboratories recorded all the necessary analyzes to obtain the results given in the mock case. It can be highlighted that only one laboratory (4%) introduced Scientific Considerations in relation to the profiles of both Autosomal and Y-Chromosome markers.

Regarding the Genetic Comparisons, 48% of the laboratories registered genetic comparisons of autosomal profiles, and only 36% indicate LR assessment as the applied method. 28% specified they used *LRmixStudio* software. More than half of the laboratories (56%) did not specify the hypotheses raised for the case, and only 12% (3 laboratories) indicated the hypotheses. With regard to Genetic Comparisons of Y-chromosome profiles, only 20% of the submitted reports (from 5 laboratories) recorded having made this type of comparisons in this section, and only one laboratory (4%) specified to have applied LR.

Date of analyses

28% of the laboratories did not record the date of the analyses, when ISO/IEC 17025:2017 sets out so (section 7.8.2.1, paragraph h) [30]. In addition, all of these recorded the start date of analyses, but only 52% of the total recorded finish date.

Notes

72% of the laboratories included additional notes in their reports. 40% of the total, referring to the custody of the remains of evidences; 28%, to the custody of the remains of extracts; and to custody in general, 28% of the laboratories. In addition, one laboratory (4%) introduced notes about if further analyses proceeds, about profiles introduction in CODIS database (4%), or other types of notes (4%) (see page 13 of Supplementary data of Appendix A5).

On the other hand, it should be noted that 32% of the laboratories included notes about autosomal markers mixture profiles, mainly referring to statistical analysis applied (20% of the total). Besides, 12% of the laboratories included notes about Y-chromosome, mainly also referring to statistical aspects (8% of the total).

Bibliography

80% of the participating laboratories included bibliographical references in their reports, as CNUFADN recommends [35]. 28% of the laboratories included a section as such, while the remaining 52% added citations throughout the body of the report. This bibliography was basically composed of: scientific articles (60%), internal procedures (36%), current regulations (6%) and websites (44%), for example, reference databases (e.g., YHRD).

Signatures

Surprisingly, not all reports included signatures of the experts who made the report, as ISO/IEC 17025:2017 clearly indicates (section 7.8.2.1, paragraph o) [30]. Only 84% of participating laboratories did it.

Other sections

ISO/IEC 17025:2017 (section 7.8.2.1, paragraph l) [30] requires that each report shall include "a statement to the effect that the results relate only to the items tested, calibrated or sampled". In addition, at the end of section 7.8.2.1, it recommends that "the laboratory should include a statement specifying that the report shall not be reproduced except in full, without approval of the laboratory". In this regard, only 40% of the submitted reports included the first statement, and only 48% of the reports introduced the declaration of total or partial non-reproduction.

Annexes and/or appendices (assessment of contents)

And finally, only 3 laboratories (12%) introduced annexes as such. Further information on the content of these annexes can be seen in the Supplementary data of Appendix A5 (page 16).

3.3.2. Evaluation of conclusions section

It is interesting to note separately the results and conclusions reported by the participating laboratories. Obviously, all of them include these two sections in their reports, with more or less complexity.

Extensive details about the content assessment of these reports sections are also included in the Supplementary data of Appendix A5.

Assessment of preliminary analysis results.

The SEMEN presence is reported.

A first point to evaluate is whether the laboratories report semen presence, based on the results provided in the exercise. 56% of the laboratories reported semen presence in the analyzed sample, although the provided data were quite determinant in this regard.

It is important the laboratory establishes the fluid source of the genetic material recovered, whenever possible. Such information may be key to the case, interpretation and assessment by Judicial Authority. It is not the same that the profile (mixture or unique) may come from a certain biological fluid (such as semen) that may come from other (e.g., saliva), or even, may come from a sampled surface of a crime scene including invisible stains (where the background, persistence, and

transfer of DNA must be considered). This information could be relevant to Court.

Evaluation of autosomal markers mixture

In relation to this assessment, more than half of the participating laboratories (15 labs) reported that at least 3 individuals contributed to the mixture, while the rest of the laboratories (10 labs) fixed the number of contributors in 3.

With respect to known contributors, whose profiles are provided among the data of the exercise, a large majority (18 laboratories) indicated in the conclusions that the "suspect" was included in the mixture. Six laboratories indicated, "It is included, although alleles are missing". And only one laboratory made no mention of the suspect's contribution to the mixture.

On the other hand, in relation to how Likelihood Ratio (LR) calculation has been performed, if it has been done, it can be indicated that a large majority of the participating laboratories (17/25, 68%) did it automatically. In addition, 2 labs performed the LR assessment, but they did not indicate how they performed the calculation. And 5 labs did not perform LR calculation. One, in particular, indicated they did not perform this calculation when faced with a complex mixture.

In relation to those who automatically performed the calculation, the vast majority (15 out of 17 labs) carried out this calculation using ***LRmixStudio*** software [15–17], 1 lab using the relatively recent ***EuroForMix*** software [18–20], and the last one using ***DNAMIX*** software [11].

Regarding LR assessment, the vast majority (17 laboratories) proposed the same combination of hypotheses (see Table 1): both victim, suspect, and regular partner have contributed to the mixture (Hp: V + S + P), versus victim, regular partner and an unknown have contributed to the mixture (Hd: V + P + U). One of these also proposed another pair of hypotheses: combination of the three known people (Hd: V + S + P), versus victim and two unknown people (Hd: V + U1 + U2) contributed to the mix. A different lab independently valued the couple contribution (Hp) against an unknown (Hd): P/U. On the other hand, another laboratory estimated contributors to the mixture were the victim and regular partner (Hp: V + P), versus victim and an unknown person (Hd: V + U). It is assumed majority mixture was valued, once the minor alleles had been extracted, although this particular one was not specified.

Finally, with respect to LR values obtained by each laboratory that make the same calculation, it must be emphasized that no laboratory reported an equal LR value among them. Obviously, it cannot be justified this circumstance due to the use of different databases, since the reference population was fixed. In any case, 4 laboratories reported more or less close values among them. However, even in these cases, its similar LR value could not be justified by obtaining the same mixture profile during edition.

It is also surprising that, although 15 laboratories used the same software (***LRmixStudio***), the range of LR values covers up to 12 orders of magnitude. Plausible explanations would range from the final allelic composition of the edited mixture profile to the parameters used in the software (*drop-in* probability; *drop-out* probability; if any sub-structuring population correction is applied).

This dispersion of LR values only highlights the need each laboratory possesses fully defined and validated its criteria for editing and interpreting mixtures, taking into account international recommendations [1–10]. These criteria should be sufficiently clear and applicable by any expert in the laboratory and should be reflected in an internal document ("Standard Operating Protocol", SOP), which should be available if Competent Authority or any part of the process required it.

Following the last GHEPMIX exercises keynote [27], it also highlights the imperative need for continuous and integrated training in software handling that the laboratory determines to use in its usual casuistry [21]. Obviously, this training implies knowing and communicating, if it is necessary, in court: how LR calculation was performed, why certain analysis parameters have been used, and the results obtained. On the other hand, the laboratory should also have validated all those software and/or tools for statistical calculation of evidence weight, in accordance with international recommendations [22].

3.3.3. Evaluation of chromosome-Y markers mixture

A large majority of laboratories (15 laboratories) did not perform any statistical analysis with Y-chromosome results. In particular, two laboratories indicated they did not perform such analyses because it was a complex mixture. The other laboratories (8 labs) did calculate LR, two of them also performed an additional evaluation, the "performance test".

In more detail, 6 laboratories applied the same combination of hypotheses: "suspect" and "regular partner" of victim contributed to the Y chromosome markers mixture (Hp: S + P); versus "regular partner" and an unknown from reference population have contributed to it (Hd: P + U). A single laboratory presents another pair of hypotheses: "suspect" and an unknown have contributed to the mixture (Hp: S + U), versus 2 unknowns people (Hd: U1 + U2).

Table 1
Hypothesis and LR values obtained by each of the participating laboratories. All laboratories used the *LRmixStudio* software, except those marked as * (*EuroForMix*) and ** (*DNAMIX*). Legend: V (Victim), S (Suspect), P (Regular partner), U (Unknown).

			Other evaluations	
Labs	LR value	Hypothesis	LR value	Hypothesis
GHEPMIX_08*	1.7200E + 02	V + S + P/V + U + P		
GHEPMIX_23	2.6000E + 03	V + S + P/V + U + P		
GHEPMIX_26	6.1640E + 03	V + S + P/V + U + P		
GHEPMIX_17	6.5565E + 04	V + S + P/V + U + P		
GHEPMIX_07	6.8487E + 04	V + S + P/V + U + P		
GHEPMIX_05	1.4800E + 05	V + S + P/V + U + P		
GHEPMIX_22	2.8776E + 05	V + S + P/V + U + P		
GHEPMIX_06	3.2224E + 05	V + S + P/V + U + P		
GHEPMIX_16	4.3423E + 05	V + S + P/V + U + P		
GHEPMIX_18	1.3900E + 06	V + S + P/V + U + P		
GHEPMIX_03	1.8200E + 06	V + S + P/V + U + P		
GHEPMIX_02	2.7323E + 06	V + S + P/V + U + P		
GHEPMIX_20	5.5183E + 06	V + S + P/V + U + P		
GHEPMIX_15	1.9820E + 07	V + S + P/V + U + P		
GHEPMIX_27	1.3587E + 08	V + S + P/V + U + P	7.4048E + 19	P/U
GHEPMIX_13**	2.7300E + 10	V + S + P/V + U + P		
GHEPMIX_10	3.2032E + 14	V + S + P/V + U + P	1.1551E + 07	V + S + P/V + U1 + U2
GHEPMIX_24			1.3400E + 19	V + P/V + U

On the other hand, up to 4 laboratories did not value the mixture as such, but each profile separately, both suspect (S) and regular partner (P). And only 2 laboratories presented frequency of suspect profile in the considered reference database.

With respect to LR values obtained for Y chromosome markers, they presented a dispersion similar to that obtained for Autosomal markers. It can be appreciated that the causes of this dispersion are not only found in the different proposed hypotheses but mainly in the reference population used to perform LR evaluation. Because of this circumstance, it is important and fundamental that population group with which we are working appears in the results issuance report. This would explain and justify possible differences in the values collected in the different expert reports for the same case.

Conclusions

It should be emphasized the vast majority (21 laboratories) did not rule out the contribution of the "suspect" to the sample submitted (vaginal swab) in their conclusions. Two laboratories did not express it explicitly and two other laboratories did not give a conclusion about it. These last two groups are striking because in the first case one can find expressions as "it is not possible to establish that the suspect is the minority contributor to the mixture". And in the second case, on account of the complexity of the mixture, "it does not allow to determine any of its components". It is surprising how, given the same results provided to the participating laboratories, one can come to report such opposite conclusions. It is complex to evaluate this point, because it depends a lot on the laboratory and, ultimately, on the criteria it has established for mixture profiles evaluation.

In addition, it is necessary to point out, since suspect contribution to the mixture is not ruled out by many laboratories, there is a certain disagreement between those laboratories which did not rule out the compatibility and those which valued the weight of evidence. As far as possible, genetic tests must be evaluated and uncertainty of the detected match must be emitted. Several International Institutions and Organizations recommend it (ENFSI [26], ISFG [2], SWGDAM [7]): whenever compatibility is detected from the genetic analysis, it must be statistically evaluated, and of course, that assessment must be reflected in the report, which is issued to the Judicial Authority.

Regarding LR expression in conclusions, 14 laboratories did not perform any type of verbalization. These include not only those that did not make an LR assessment but also some of those that did it. Of the rest that made the LR assessment, 10 laboratories made a correct verbalization, without conditional transposition. And only one laboratory verbalized LR in a strange way, but in no case, incorrect.

4. Conclusions

After six editions of this exercise, the differences in the editing and interpretation of a same mock mixture profile by GHEP-ISFG laboratories are still evident. However, it is noteworthy that with regards to allelic assignments of the mixture components, an evolution was noticeable over the preceding editions [27]. In this last edition of the exercise, there are not any participating laboratories would perform an allelic assignment manually and only one laboratory made an exclusively automatic assignment in the routine casework. Besides, it is important to highlight the growing trend to validate this type of analysis with respect to previous editions [27], despite "controlled" samples used in this kind of studies cannot accurately reflect the reality of all samples received in forensic cases [27]. However, generic approaches are possible (by considering a number of major/minor contributors) to deal progressively with the most common samples received by each laboratory, until to reach the ideal point where if a rare profile is obtained, a validation procedure can be available to simulate the obtained situation with the aim to validate such rare results.

Continuing with this idea, as it was already indicated in previous editions [27], it is necessary a correct and complete validation to characterize a profile as a mixture. We already emphasized that the use of thresholds for carrying out the evaluation of genetic profiles is not risk-free and it may sometimes lead to erroneous conclusions [6,7,36]. In spite of this, the use of thresholds helps laboratories make decisions when issuing a conclusion on a genetic profile.

With respect to the transmission of results through a report, previous studies already observed differences between the reports issued by the participating laboratories [24]: extensiveness of the reports, explanations of technical issues, the use of explanatory appendices, level of reporting, use of context information, and, most markedly, the type and content of the conclusions. Participating laboratories belonging to GHEP-ISFG behaved similarly in this collaborative exercise. But in addition, with rare exceptions, the reports issued by these laboratories did not follow the ISO/IEC 17025:2017 requirements. As we upheld in previous studies [27], the training stage in the forensic field is essential in order to thrive and transmit with certainty the result of the analysis to the courtroom.

Regarding statistical assessment, those laboratories that perform it, use the LR value as the statistical parameter [2]. In this exercise, the hypotheses were left open to enable the calculation of the LR value, according to each laboratory's criteria. In this sense, detailed knowledge of the background of the case will certainly allow adjusting more accurately the assumptions to work on [27]. Although in any case, it seems more advisable to offer to the judge different possibilities of assumptions and hypotheses, unless they have already been indicated by the prosecutor or the defense. Taking all this into account, the obtained results were much dispersed and quite varied. Since allelic frequencies were set by the organizers, one of the explanations of this fact could be the use of different software to calculate LR. However, as we have seen above, the vast majority of those who do the LR assessment use the same software, ***LRmixStudio*** [16,17]. So, apart from small differences as a consequence of different internal software parameters (*drop-out*, *drop-in*, etc.), an important source of LRs variation could be the profile edition (e.g., application of different *stutter* thresholds). In addition, an inadvisable practice that has been observed in some laboratories consists of editing the challenged profile taking into account the reference profiles, as it involves a biased edition. At this point, it is evident the importance of a continuous training, not only in the edition and interpretation of mixture profiles, but also in the use of new computer tools that are continuously being developed [16,18], and which must be validated in the laboratory according to international recommendations [22]. In addition, understanding what software exactly does, it is essential for a correct results transmission to Courts.

The need for this training has been continuously pointed out by several international institutions and organizations (NIST, ENFSI, ISFG, GHEP-ISFG), which have been permanently contributing to this respect in the recent years. With regard to the issuance of reports, some international [25,26] and national [35] organizations have also developed guidelines on how to express the results, always conforming to ISO/IEC 17025:2017 [30]. In whatever case, the main idea that should remain is the importance of transmitting the conclusions in the reports in a manner sufficiently intelligible for the Court: clear and concise ideas, avoiding the conditional transpositions, and mainly, avoiding ambiguities.

5. Conflict of interest statement

None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fsigen.2018.05.005.

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