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Results of an inter and intra laboratory exercise on the assessment of complex autosomal DNA profiles

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ABSTRACT

The interpretation of complex DNA profiles may differ between laboratories and reporting officers, which can lead to discrepancies in the final reports. In this study, we assessed the intra and inter laboratory variation in DNA mixture interpretation for three European ISO17025-accredited laboratories. To this aim, 26 reporting officers analyzed five sets of DNA profiles. Three main aspects were considered: 1) whether the mixed DNA profiles met the criteria for comparison to a reference profile, 2) the actual result of the comparison between references and DNA profiling data and 3) whether the weight of the DNA evidence could be assessed. Similarity in answers depended mostly on the complexity of the tasks. This study showed less variation within laboratories than between laboratories which could be the result of differences between internal laboratory guidelines and methods and tools available. Results show the profile types for which the three laboratories report differently, which informs indirectly on the complexity threshold the laboratories employ. Largest differences between laboratories were caused by the methods available to assess the weight of the DNA evidence. This exercise aids in training forensic scientists, refining laboratory guidelines and explaining differences between laboratories in court. Undertaking more collaborative exercises in future may stimulate dialog and consensus regarding interpretation. For training purposes, DNA profiles of the mixed stains and questioned references are made available.

1. Introduction

The interpretation of mixed autosomal DNA profiles comprises a prominent part in the field of forensic genetics. In the past two decades, several recommendations and guidelines have been presented to aid in this interpretation (*e.g.* [1–9]). Interpretation strategies have changed over time with evolving technologies that include, for instance, more sensitive STR typing kits, enhanced interrogation techniques and biostatistical systems to compute a weight of evidence (WoE) for samples with stochastic effects, such as drop-out and drop-in. Despite such advancements, not all crime stain profiles are suitable for WoE evaluation [10,11] or even for comparison to a reference DNA profile. There are no paramount guidelines describing which profiles are suitable for comparison and/or biostatistical analysis; these depend on casework practice, juridical context and available methods in each laboratory.

Previous studies comparing mixture interpretation within and among laboratories demonstrated that participants could correctly identify major-contributor alleles in mixed samples [12], but that the interpretation of minor-contributor alleles varied significantly between laboratories [13]. Large mixture interpretation studies undertaken by the National Institute of Standards and Technoloy (NIST) also showed there can be a wide range of results within and between laboratories for profile comparisons and reported statistics, which could be attributed to the use of different protocols, instruments with different sensitivities, and possibly experts having different levels of experience and training [14–16]. Studies on biostatistical calculations for complex mixtures showed similar answers when the same likelihood ratio model was applied to the same set of hypotheses, but varied when reporting scientists had to define the hypotheses themselves [17,18] and/or used a different model [15] or assigned the number of contributors [19]. Since inconsistencies in reporting between laboratories seem to be currently unavoidable for highly complex samples, it is important to assess the level of inconsistency both within and between laboratories, share such data [20] and advance interpretation tools [3,9,18,21,22], as this will aid training forensic scientists, improving laboratory guidelines and explaining differences in court. Here, we contribute to this concept by examining the inter and intra laboratory variation for three European ISO17025-accredited laboratories using profile sets of varying complexity. We address three main aspects: 1) is a profile

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regarded suitable for comparison to a reference DNA profile, 2) what is the result of the comparison and 3) is the comparison regarded suitable for weight of evidence (WoE) evaluation.

2. Materials and methods

2.1. Sample preparation

DNA mixtures were prepared using commercially available pristine DNA extracts (hDNA, 9947A and DNA007, all Life Technologies, Nieuwerkerk aan den IJssel, The Netherlands), DNA extracted from buccal swabs as described in [12,23] and DNA extracts selected from a reference set of 2085 Dutch males [24,25]. In total ten DNA mixtures were prepared as presented in Table 1.

The DNA mixtures were amplified in fourfold, except for samples 2A and 2B that were amplified once, using the Next Generation Multiplex (NGM) kit (Life Technologies) at 29 cycles. The manufacturer's recommendations were followed except that the total DNA input varied from 24 pg to 1750 pg and that mixed samples were used. Replicates were prepared simultaneously and amplified at the same time on a PCR micro-titre plate and 9700 thermal cycler (Life Technologies). Amplification products were separated by capillary electrophoresis (CE) on the same 3130xl Genetic Analyzer (Life Technologies) using either 3 kV 5 s (samples 1–4, A and B) or 9 kV 10 s CE injection settings (samples 5A and B). Prior to 9 kV 10 s CE injection, PCR products were de-salted using Performa® DTR filtration columns as described by the manufacturer (Edge Bio). For 3 kV 5 s CE injection, 0.3 μl internal standard (LIZ500), 8.7 μl HiDi Formamide (both Life Technologies) and 1 μl of PCR product or allelic ladder were mixed. With enhanced injection, 0.3 μl diluted internal standard (LIZ500 1:100 in HiDi formamide), 8.7 μl HiDi Formamide and 2 μl of purified PCR product or allelic ladder (1:20 diluted in water) were mixed.

DNA profile analysis was carried out using GeneMapper ID-X version 1.1.1 (Life Technologies). We applied a detection threshold of 50 relative fluorescence units (rfus). Locus specific backward (−1 repeat unit) and forward (+1 repeat unit) stutter filters, as determined by in-house validation [26], were applied to remove stutter peaks. The in-house determined stochastic threshold corresponding to the used CE injection setting was also provided (Table 1).

2.2. Participants and lay-out of the collaborative exercise

Twenty-six reporting officers (ROs) of three different ISO17025-accredited European forensic laboratories participated in this paper challenge. These three laboratories reflect north-west European laboratories and are not representative of all forensic laboratories across the world. We do, however, provide the mixed stains and the reference profiles as provided to our participants (Supplemental material 1 (set A) and Supplemental material 2 (set B)) enabling other forensic laboratories to compare performances. Each RO performed five tasks that included the evaluation of the DNA samples in either 'set A' or 'set B' (Table 1: set A comprises sample sets 1A, 2A, 3A, 4A, 5A; set B comprises sample sets 1B, 2B, 3B, 4B, 5B). Sample information presented to the participants were the total amount of DNA per replicate, the settings for PCR and CE, the applied detection threshold and stutter filters, the stochastic threshold, and whether a reference profile for a person of interest (POI) and/or known contributor were available. Although PDF, .csv and .ser files were provided, only the PDF files were used by the participants.

The DNA profiling data were interpreted individually, without collegial review. This departs from the laboratories' regular casework policy, but allowed for assessment of intra laboratory variation. Participants completed an online questionnaire for each of the five tasks and considered the DNA profiling data as if they were the only DNA evidence in the case; rework could not be performed and taking new samplings was deemed not possible. The questionnaire included questions regarding the number of contributors, presence of a major contributor, meeting the criteria for comparative analyses and weight of evidence calculations. Each participant was free to use the methods/tools of their, or their laboratory's choice. The numbers of contributors were estimated based on allele counting (maximum number of alleles per locus and/or total number of unique alleles per profile) and/or expert opinion. Inference of the major contributor's alleles was done manually or using the LoCIM-tool [12]. If the mixture met the criteria for comparison, and the POI was included, weight of evidence could be determined. For computation of the random match probability (RMP), the random man not excluded (RMNE) or a standard likelihood ratio (LR), participants used their in house tools for which we did not ask to specify details. For some samples, laboratory C participants computed the LR using the semi-continuous model LRmix Studio version 2.0.1 (lrmixstudio.org). Participants used their own allele frequency data or the allele frequency data provided by the organizing laboratory [25].

3. Results

3.1. Participants characterization

In this study, 26 ROs participated: ten from laboratory A, five from laboratory B and 11 from laboratory C. Over the laboratories, the years of experience in profile assessments was 2–5, 5–10 and >10 years for 15%, 58% and 27% of the participants, respectively. ROs from laboratory B were not used to assessing NGM DNA profiles, while ROs from laboratory A were not used to examining profiles obtained using enhanced interrogation, such as the samples in task 5. Standard practice in laboratories A and B is analysing up to two replicates, while in four

Table 1
Description of the ten profile sets used in this collaborative exercise.

Sample number	Mixture proportion	DNA input PCR (pg)	Number of NGM replicates	3130xl CE injection settings	Stochastic threshold	Person of interest	Known contributor provided
1A	10:1:1	300:30:30	4	3kV5s	175 rfus	300 pg contributor	–
1B	10:1:1	300:30:30	4	3kV5s	175 rfus	300 pg contributor	–
2A	2:2:1:1:1	500:500:250:250:250	1	3kV5s	175 rfus	500 pg contributor 2	500 pg contributor 1
2B	2:2:1:1:1	500:500:250:250:250	1	3kV5s	175 rfus	500 pg contributor 2	500 pg contributor 1
3A	5:1:1:1	150:30:30:30	4	3kV5s	175 rfus	150 pg contributor	–
3B	5:1:1:1	150:30:30:30	4	3kV5s	175 rfus	150 pg contributor	–
4A	5:1:0.2	150:30:6	4	3kV5s	175 rfus	Non-contributor	–
4B	5:1	150:30	4	3kV5s	175 rfus	Non-contributor	–
5A	1:1	12:12	4	9kV10s + de-salting	Not determined, but expected >2000 rfus	12 pg contributor 1	–
5B	1:1:1	9:9:9	4	9kV10s + de-salting	Not determined, but expected >2000 rfus	9 pg contributor 1	–