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(Article begins on next page)

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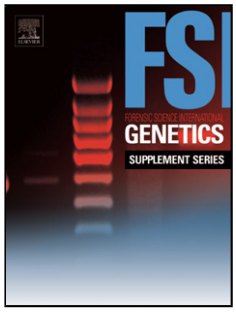

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An alternative application of the consensus method to DNA typing interpretation for Low Template-DNA mixtures

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Abstract

Aim of this study is to define a rigorous approach to Low Template - DNA mixture interpretation; our laboratory adopted two different models, i.e. the semi-continuous method (with the use of LRmix Studio and Lab Retriever software) and the fully continuous method (with the use of DNA•VIEW^{TM} software). When approaching caseworks where complex DNA profiles are involved, evidence evaluation may heavily affect the outcome of a Trial so extreme caution is needed in order to achieve correct results' interpretation. As consequence of this, a conservative "statistic consensus method" was developed and validated on simulated LT-DNA mixture and then applied in real casework. In particular, our statistic consensus approach consists in comparing likelihood ratio values provided by different software and, only if results turn out to be convergent, the most conservative likelihood ratio value is finally reported. On the contrary, if likelihood ratio results are not convergent, DNA interpretation is considered inconclusive.

Keywords: LT DNA; mixture interpretation; semi-continuous model; fully continuous model; LRmix Studio; Lab Retriever; DNA•VIEW^{TM}

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Introduction

The interpretation of Low Template - DNA (LT DNA) mixtures collected during crime scenes investigations represents one of the most challenging situation for forensic scientists and analysts[1,2]. Despite several recommendations have been suggested over the past years concerning the importance of evaluating factors that may affect inclusion or exclusion hypotheses from prosecutor or defense, a rigorous approach has still not been defined in order to establish a "universally-accepted" methodology. As a consequence, this lack of regulation leads experts to differently interpret evidence in the Court applying several statistic approaches, involving the chance of making judgements "beyond any reasonable doubt" even more difficult. As largely reported in literature, three main models (*i.e.* binary, semi-continuous and fully continuous) can be used for interpretation of DNA profiles obtained from samples involving DNA mixtures templates. These models present different degrees of complexity in terms of application and comprehensibility. The most comprehensible model is the binary one[3]. However, it is broadly accepted that binary models are not suitable for complex mixtures and LT DNA evaluations, since they do not take into account several important parameters and stochastic effects such as drop-out and drop-in and, above all, thresholds (in terms of limit of detection/analytical threshold and limit of quantitation) and peak heights data. For this reason, semi-continuous [4,5] and fully continuous models[6] of evidence evaluation were developed. In fact, our interpretative approach is based on the simultaneous application of both interpretation models. The fully continuous model is considered the most powerful one but its application is more difficult to be explained and accepted in Courtrooms. Fully continuous models necessarily require a computer software and if analysts are not sufficiently trained, they may not observe model's limitations using then fully continuous approach in wrong contexts. Consequently, semi-continuous approach is widely applied since it is more comprehensible and its workings are less complex than fully continuous' ones. However, it does not take into account peak height data, thus removing a piece of information that could be extremely useful, particularly when interpreting LT DNA complex mixtures. Due to the fact that extreme caution is needed to achieve a correct interpretation of the DNA results, our laboratory decided to adopt a so-called "*statistic consensus approach*". As consensus method itself, which makes use of alleles observed in different replicates, our approach compare LR values from cited software and the most conservative one is reported if LR results turn out to be similar and convergent. On the contrary, if LR results are not similar, interpretation process provides an inconclusive decision. After the "*statistic consensus approach*" has been validated on simulated samples, it has been applied to real cases in order to highlight the difficulties to reach a clear

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conclusion when considering a large number of unpredictable parameters relative to LT DNA mixture evaluation.

Material studied, methods, techniques

Software

LRmix Studio and Lab Retriever are both open-source and free of charge software dedicated to the semi-continuous approach. LRmix Studio is an updated version of LRmix software, a module included into R package *forensim* from Comprehensive R Archive Network (CRAN). LRmix Studio v1.0.2. was downloaded from website http://www.lrmixstudio.org/. Lab Retriever v2.2.1 was downloaded from Scientific Collaboration, Innovation and Education (SCIEG) website (http://scieg.org/lab_retriever.html). Conversely, DNA•VIEW™ is a commercial software developed by C.H. Brenner (http://dna-view.com/) and was adopted to interpret mixture DNA profiles by means of fully continuous model (v34.21 was adopted).

DNA extraction, amplification and detection laboratory protocols were fully validated and accredited according to ISO 17025 and Scientific Working Group on DNA Analysis Methods (SWIGDAM) recommendations. GeneMapper®ID-X v1.4 (from Life Technologies), OSIRIS v2.4 (from http://www.ncbi.nlm.nih.gov/projects/SNP/osiris/) and ArmedXpert™ v3.0.7.999 (from NicheVision Forensics LLC, http://www.armedxpert.com/) were employed for this purpose.

Statistic consensus approach

Comparisons of both semi-continuous' and fully continuous' results were performed within the "*statistic consensus approach"* in order to simultaneously exploit the advantages of both interpretation techniques. Practically, our approach compared all LR values from different software and, if they were similar and convergent, analysts reported the lowest one. In contrast, if LRs were not coherent, DNA interpretation process was reported as inconclusive. This approach was validated, too; in particular, several two-persons mixtures (in proportions of 19:1, 9:1, 6:1, 4:1, 2:1, 1:1, 1:2, 1:4, 1:8, 1:9, 1:19) and three-persons mixtures (in proportions of 20:9:1, 8:1:1, 6:3:1, 1:1:1) were analysed in and amplified with 9 different amplification kits (from Life Technologies and Promega). Statistic consensus approach showed LR results indicating that the probability of observing the evidence was more likely if inclusion hypothesis was true (investigated subjects contributed to the samples) compared to exclusion hypothesis (unknown subjects contributed to the samples); moreover, LR results were coherent and convergent for all mixtures and analytical amplification kits (NIST U.S. population dataset was adopted as reference database[7]).

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Results & Discussion

Statistic consensus approach was adopted for evidence interpretation of several real caseworks and one of them is reported in this study. In particular, a subject (S) was identified during investigation activities as suspect of a committed theft; his genetic profile was compared to several genetic profiles recovered on different objects that were found on the crime scene. Several matches between the suspect and the evidenced biological samples were calculated; significant LR results are reported in Table 1. Statistic interpretation provided LR values that strongly supported H(p) hypothesis for both interpretative models. Consequently, probability of observing the evidence was more likely if H(p) was true compared to H(d); final decision supporting prosecutor hypothesis was provided "beyond any reasonable doubt", as suggested by developed "statistic consensus approach".

Conclusions

As a pilot study, applicability to LT DNA interpretation of the developed *"statistic consensus approach"* was demonstrated. In this study, both semi- and fully continuous methods are applied in order to give an unbiased interpretation to the cases. In our opinion, one of the main advantages of this method is that it is extremely conservative. The comparison of results obtained exploiting different software, together with rigorous internal validation procedures, allowed us to confirm specific H(p) conclusions "beyond any reasonable doubt". The *"statistic consensus approach"* helped us to highlight the difficulties in evaluating the profiles obtained. Though the application of several software and different models is questioned, our approach may be explained and accepted by Courts more easily, since it combines different advocated issues such as judgement's conservatism, together with semi-continuous models' comprehensibility and fully continuous models' complexity.

New studies are investigating reasons of divergent results trying to evaluate which parameters can suggest the use of most advanced continuous methods alone when managing particularly challenging samples.

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Conflict of interest statement

The authors of this manuscript certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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References

[1] D. Balding, Evaluation of mixed-source, low-template DNA profiles in forensic science, J. Natl. Acad. Sci. 110 (2013) 12241–12246.

[2] P. Gill, H. Haned, A new methodological framework to interpret complex DNA profiles using likelihood ratios, For. Sci. Int. Genet. 7 (2013) 251–263.

[3] T.W. Bille, S.M. Weitz, M.D. Coble, et al, Comparison of the performance of different models for the interpretation of low level mixed DNA profiles, Electrophor. 35 (2014) 3125–3133.

[4] H. Haned, Forensim: An open-source initiative for the evaluation of statistical methods in forensic genetics, For. Sci. Int. Genet. 5 (2011) 265–268.

[5] K.E. Lohmueller, N. Rudin, Calculating the Weight of Evidence in Low-Template Forensic DNA Casework, J. For. Sci. 58 (2013) 243-249.

[6] M.R. Wilson, In response to "Comparison of the performance of different models for the interpretation of low level mixed DNA profiles," Electrophor. 35 (2014) 489–494.

[7] C.R. Hill, D.L. Duewer, M.C. Kline, M.D. Coble, J.M. Butler, U.S. population data for 29 autosomal STR loci, For. Sci. Int. Genet. 7 (2013) e82–e83.

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Table

Table 1. LR values and results relevant to real casework (U stands for unknown subject(s)).

Samples	**A**	**B**	**C**	**D**	**E**
Description	Sample recovered on backpack nr.1	Sample recovered on backpack nr.2	Sample recovered on a sweatshirt	Hair samples recovered on a swetshirt	Hair samples recovered on a cap
	Hypothesis				
H(p)	S + 2U	S + 1U	S + 2U	S + 1U	S + 1U
H(d)	3U	2U	3U	2U	2U
Software	**Likelihood Ratio (LR)**				
LRmix Studio	1.29E+0	1.85E+14	2.41E-2	1.33E+13	9.15E+8
Lab Retriever	1.20E+0	1.89E+14	2.12E-2	1.21E+13	1.07E+9
DNA•VIEW™	1.09E-14	4.66E+11	2.24E+8	3.45E+15	5.72E+20
Interpretative decision	**Inconclusive**	**Support to H(p)**	**Inconclusive**	**Support to H(p)**	**Support to H(p)**