



Report on investigation regarding general concerns about DNA mixture interpretation

November 19, 2014

Introduction

Concerns about the Department of Forensic Sciences' Forensic Science Laboratory Forensic Biology Unit ("the Unit") were expressed by the District of Columbia's United States Attorney's Office (USAO) on 12 SEP 2014, specifically about the Unit's method for interpreting DNA profiles in mixed samples and the application of appropriate statistics to aid in the assessment of the significance of including a person in the mixture.

Background

A DNA sample is "mixed" when more than one contributor can be identified in the sample based on the apparent genetic profile present. The amount of DNA contributed by the sources will vary and this may affect the interpretation of the results. Three main statistical methods are used for determining the significance of including a person in the mixture, Combined Probability of Inclusion (CPI), Random Match Probability (RMP), or Likelihood Ratios (LR); the Department of Forensic Sciences (DFS) Forensic Science Laboratory Division uses the CPI method. These analyses are all conducted in the DFS Forensic Science Laboratory Division's Forensic Biology Unit.

Issue or allegation

An expert for the USAO expressed general concerns to DFS on 12 SEP 2014 regarding the Unit's protocol for interpreting mixed DNA profiles, the calculation of appropriate statistics, and the CPI method upon which the Unit's protocol was based.

Response

The issue was presented to the DFS Science Advisory Board (SAB) on 7 OCT 2014. The SAB Chair assigned a group of 4 individuals on the SAB with experience in forensic biology and statistics to review the Unit's protocols. The SAB reviewed the DNA mixture interpretation protocols and found them to be adequate but offered a list of 12 recommendations to enhance the existing protocols (Appendix A). A conference call was held on 4 NOV 2014 with the USAO's expert, USAO personnel, members of the SAB, and DFS personnel wherein all agreed that the Unit's current protocol was adequate but could be enhanced.

Discussion

Of the 12 recommendations, some were already in place in other of the Unit's protocols and others were already in the process of being incorporated into a new protocol.

Outcomes and Actions

All of the recommendations from the SAB will be incorporated into DFS protocols that are estimated to be in place by end of January 2015. Any cases going to trial between the date of this report and the end of January that involve mixtures that require calculations of significance of inclusion will either require a request for continuation until the protocols are in place, and the

calculations can be conducted under the new protocol, or, if no continuance can be obtained, reports will be issued under the current protocol.

Appendix A: DNA Mixture Interpretation Recommendations, dated 5 NOV 2014

November 5, 2014

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As requested, a four member panel selected from the Scientific Advisory Board reviewed the procedures of the DFS Forensic Science Laboratory regarding the interpretation of DNA mixtures. The following comments and recommendations are offered based on the review of the following documents:

1. FBS15 – Identifiler Plus Interpretation Guidelines, revision 3, dated 11/26/2013;
2. FBS18 – Population Statistics, revision 5, dated 11/26/2013;
3. Power Point presentation entitled “BruceBudowleMixturesDFS Issues” (undated) provided via email on 10/16/2014 from Michael Ambrosino;
4. SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories, dated 1/14/2010.

The two standard operating procedures reviewed were found to be well written but generic and quite limited in scope. While they may provide minimal adequate guidance for the interpretation of high quality single-source or two-person mixed DNA profiles with no allele drop-out, there is a lack of specificity and detail in several important areas relevant to current issues in the

interpretation of low template DNA¹ and DNA mixtures. Several of the relevant issues were outlined in the presentation from Dr. Budowle. It is advised that the laboratory modify and expand the existing procedures based on published literature and in-house validation studies using appropriate samples of known origin to provide more specific guidance and information to address the issues resulting from stochastic effects and the interpretation of DNA profiles resulting from DNA mixtures with two, three or more contributors. Where applicable, the modifications should be in compliance with the current SWGDAM Interpretation Guidelines. Some suggestions for areas to include in the revisions are:

1. State the analytical threshold and stochastic threshold to be used and under which conditions (e.g., low template vs. high quality profile, amplification cycle number, injection voltage, injection time, etc.).

2. Detection, analysis and interpretation of DNA profiles resulting from the amplification of single- source low template DNA¹, including criteria for the inclusion and exclusion of known individuals, and the appropriate method(s) for statistical frequency calculations.
3. Information for assessing the possible number of contributors in a mixed DNA profile and how to use that information in the interpretation of the profile and the generation of statistical frequencies.
4. Detailed explanation of how to interpret two-person mixtures, including criteria for determining a major/minor two-person mixture and how to resolve a mixture assuming the presence of one known contributor.
5. Detailed explanation of how to interpret mixtures of three or more contributors, whether a major contributor can be assessed from a complex mixture, and if so, when. Specific treatment of profiles with suspected low template DNA and the possibility of stochastic events affecting the profile should be clearly detailed.
6. Inclusion and exclusion criteria for two, three and more contributor DNA mixtures.
7. Criteria for making a statement of “inconclusive.”
8. Statement of the software package(s) used with appropriate references for the software and associated validation studies.
9. Detailed explanation of how to calculate statistical frequencies incorporating the issues associated with low template DNA, stochastic effects and/or complex mixtures.
10. How and when to use the calculation of $2p$ vs. p^2 .
11. How to use the assumed number of contributors to assess the feasibility that all alleles from all contributors are present in the profile and when it is appropriate (and inappropriate) to use the CPI or CPE calculation.
12. How to use the stochastic threshold, stutter peak ratios, peak height ratios and mixture ratios in

¹ Low template DNA is defined here as any limiting amount of DNA (whether due to a small amount of input DNA, degradation, inhibition or any other process) that will likely result in an incomplete or altered DNA profile due to the occurrence of one or more stochastic effects during polymerase chain reaction amplification.

DNA mixtures and to incorporate possible stochastic effects, shared alleles, possible alleles in the stutter position that may be typical stutter vs. elevated stutter vs. stutter plus an allele from a minor contributor into the interpretation of the results and the calculation of statistical frequencies.

Please consider these recommendations from the Scientific Advisory Board as you review the results of your DNA mixture cases.

Irvin B. Litofsky
Chairman, Scientific Advisory Board

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