Interpretation of Complex Forensic DNA Mixtures

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Forensic evidentiary samples routinely contain DNA from multiple contributors. The interpretation of these mixtures can be a challenging task for the DNA scientist. Several approaches are discussed (no calculation- qualitative statement; probability of exclusion; likelihood ratio estimates; presumptive genotype assignment based on peak heights), which have been employed to assess the significance of an inclusion/match when DNA mixtures have been detected in casework samples. These statistical approaches are discussed in light of technical challenges that can arise when evaluating evidentiary samples.

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The detection and interpretation of mixtures is a routine, yet often challenging aspect of forensic DNA analysis. This process can be complicated by a number of factors, including possible allele overlap among an unknown number of contributors, 3-allele patterns, stochastic fluctuation with low quantity or degraded template, the semi-quantitative activity of Taq polymerase, and microvariants in primer binding sites. These technical issues can make genotype assignment and subsequent statistical evaluation of the results a challenging task for the forensic scientist. Several of these areas will be discussed.

Template Considerations

Factors such as degraded and mixed samples, ubiquitous in the forensic environment, constitute substantial interpretational challenges for the DNA analyst. Sample degradation can change the effective template quantity across the allele size spectrum. This increases the prospect for allele and locus dropout, especially for the larger amplicons. Limitations to the ability of common human DNA quantitation methods to measure DNA recovery from degraded samples can also affect the short tandem repeat (STR) data. Furthermore, even with an accurate measurement of the total amount of DNA recovered, the quantity of template from the minor component could be in the stochastic range. This can lead to greater peak imbalance of the minor profile and at least one true allelic peak being below the individual laboratory allele reporting threshold at one or more loci.

Possible stochastic effects with low template concentration should not be underestimated. Occasionally we have observed profiles from highly degraded/low DNA quantity samples where one amplification kit detects multiple contributors, whereas the other system does not. Furthermore, there have been many cases where the minor component(s) have not been detected at each of the loci examined.

In addition to the technical challenges in detecting and interpreting forensic DNA mixtures, statistical issues have been central to the adjudication of certain criminal cases (e.g., Michigan v. Coy II, 2000) (1).

Common approaches to the interpretation of forensic DNA mixtures are listed in Table 1. Advantages and limitations of these methods are discussed below in light of the technical challenges surrounding forensic DNA analysis. The DNA Advisory Board (DAB) has recently issued clarification of certain statistical issues pertaining to forensic DNA analysis (3).

No Calculation – Qualitative Statement

Some laboratories in the U.S. have not proffered a mixture statistic in sworn courtroom testimony. In such cases, the analyst might simply testify that the

Table 1. Approaches to statistical evaluation of DNA mixtures

No calculation-quantitative statement
Match probability estimation after deducing genotypes
Exclusion probabilities
Likelihood ratio calculation

evidentiary sample represents a DNA mixture and that the suspect/victim can be either included or excluded as a potential contributor to the evidentiary stain. While the lack of a statistical interpretation on a written case report is perfectly acceptable (e.g., DAB standards section 11.1.2) (4), some U.S. courts have found that sworn testimony must include a statistical assessment of the evidence:

"We ...hold that DNA evidence is only admissible when both the evidence of a match and the statistical significance of the match are admissible. Thus, we reject the State's overly simplistic argument that statistics go simply to the weight, not the admissibility of the DNA matching evidence." (1).

"[W]ithout the necessary statistical calculations, the evidence of the match was "meaningless" to the jury and, thus, inadmissible." (*Nelson v State*, 628 A2d 69, 76 [Del, 1993].)

"Without the probability assessment, the jury does not know what to make of the fact that the patterns match: the jury does not know whether the patterns are as common as pictures with two eyes, or as unique as the Mona Lisa." (*US v Yee*, 134 FRD 161, 181 [ND Ohio, 1991]).

The courts have also regularly cited the following logic in National Research Council (NRC) reports regarding forensic DNA evidence:

"Many courts have held that unless the finding of a match is accompanied by some generally accepted or scientifically sound profile frequency or probability estimate, no testimony about DNA testing is admissible. The insistence on quantitative estimation has been fueled by the observation in the 1992 NRC report (page 74) that "[t]o say that two patterns match, without providing any scientifically valid estimate (or, at least, an upper bound) of the frequency with which such matches might occur by chance, is meaningless."

"Certainly, a judge's or juror's untutored impression of how unusual a DNA profile is could be very wrong. This possibility militates in favor of going beyond a simple statement of a match, to give the trier of fact some expert guidance about its probative value." (2).

Inferring Genotypes of Contributors

A second common strategy for mixture interpretation involves deducing the genotypes of the contributors based on relative peak height proportions followed by calculating a point estimate of the combined match probability for the major or minor profile. Although this practice has been successful (especially with intimate samples), the findings of two STR inter-laboratory mixture studies suggest caution may be needed with this approach in some cases (5,6). In these studies consisting of 44 and 7 laboratories, respectively, the participants correctly detected all alleles present in the sample mixtures. However, efforts to infer the genotypes of the contributors were not always successful (incorrect genotype of major profile 10-13%; incorrect minor component 13-33%).

In addition to the interpretational challenges discussed previously, there are other factors that should

be considered. These factors include potential allele sharing among close relatives (7) and the possibility of additional contributor(s) whose alleles could be masked (e.g., by the major profile). Moreover, minor profiles may be evident only at some of the loci tested.

Attempts to analyze low level mixtures (especially from degraded samples) raise the question of how to interpret the absence of an "expected" allele. Consider the following case: an intimate swab (sperm-rich fraction) was typed with the Combined DNA Index System 13 STR loci. Minor peaks were detected (just above threshold) at 4 loci, which could not have come from the victim but which could have come from the suspect. The suspect's profile, if present, would be masked by the major profile at two additional loci. Only the victim's alleles were evident at the remaining 7 loci. Although there are several strategies for interpreting such data, a conservative stance is suggested.

Calculation of Exclusion Probabilities

A third approach for assessing complex DNA mixtures is to report the combined probability of exclusion (CPE) from an observed mixture. The CPE provides a straightforward, conservative, yet still highly informative calculation that avoids potential pitfalls associated with extrapolating the genotypes of contributors. Furthermore, it does not require knowledge of any known profiles, and it involves no assumptions about the number of contributors to the sample (8,9). In its most conservative form, the CPE suffers from reduced statistical power in not utilizing all of the data.

Calculation of Likelihood Ratio Estimates

A fourth strategy for mixture interpretation involves calculating likelihood ratio (LR) estimates. LRs provide a sound method for comparing the probability of observing genetic data under two (or more) alternate hypotheses (10). Although LRs make full use of available genetic data, determination of which hypotheses to consider is not necessarily straightforward. LR estimates involve assumptions about the identity and number of contributors to the mixture. Consequently, multiple calculations are sometimes required. "[Some] LR calculations and interpretations can be complicated, and their significance to the case may not be apparent to the practitioner and the trier of fact." (3). LR calculations are in widespread use in much of Europe for forensic interpretations and in the U.S. for paternity testing.

Conclusion

The process of mixture interpretation can be relatively straightforward or quite complex, involving laboratory and statistical considerations. While many of the technical challenges are routine features of forensic laboratory analysis and therefore cannot be eliminated, use of conservative statistical methods should obviate court objections and reduce the difficulties in mixture genotype assignment.

References

1 State of Michigan Court of Appeals. People v. Coy II. Calhoun Circuit Court, LC No. 98-001925 FC. (November 17, 2000)

2 National Research Council Committee on DNA Forensic Science. An update: the evaluation of forensic DNA evidence. Washington (DC): National Academy Press; 1996.

3 U.S. Department of Justice. Federal Bureau of Investigation. DNA Advisory Board. Statistical and population genetic issues affecting the evaluation of the frequency of occurrence of DNA profiles calculated from pertinent population database(s). 2000 Feb 23. Forensic Science Communications 2000;2(3). Available at: *http://www.fbi.gov/hq/lab/fsc/backissu/july2000/dnast at.htm*. Accessed: March 15, 2001.

4 U.S. Department of Justice. Federal Bureau of Investigation. DNA Advisory Board. Quality assurance standards for forensic DNA testing laboratories. 1998 Oct 1. Forensic Science Communications 2000;2(3). Available at: *http://www.fbi.gov/hq/lab/fsc/backissu/july2000/co dis2a.htm*. Accessed: March 12, 2001.

5 Kline MC, Redman JW, Duewer DL, Reeder DJ. Results from the 1999 NIST mixed-stain study #2: DNA quantitation, differential extraction, and identification of the unknown contributors. In: Promega Corporation. Proceedings of the 10th International Symposium on Human Identification; 1999 Sep 29 - Oct 2; Orlando, Florida, USA.

6 Ladd C, Yang NCS, Lee HC. STR Inter-laboratory mixture study. In: Proceedings of the American Academy of Forensic Sciences 53rd Annual Meeting; 2001 Feb 19-24; Seattle, Washington, USA.

7 Bourke MT, Ladd C, Yang NCS, McGilvray MK, Lee HC, Bieber FR. Sib pair identity at multiple STR loci: implications for interpretation of forensic DNA casework. In: Proceedings of the American Academy of Forensic Sciences 52nd Annual Meeting; 2000 Feb 21-26; Reno, Nevada, USA.

8 Devlin B. Forensic inference from genetic markers. Statistical Methods in Medical Research 1992;2:241-62.

9 Ladd C, Lee HC, Bieber FR. Probability of exclusion estimates in forensic analysis of complex DNA mixtures. In: Proceedings of the American Academy of Forensic Sciences 52nd Annual Meeting; 2000 Feb 21-26; Reno, Nevada, USA.

10 Evett IW, Weir BS. Interpreting DNA evidence. Sunderland (MA): Sinauer Press; 1998.

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