DNA mixture interpretation principles: insights from the NIST scientific foundation review

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ABSTRACT

The National Institute of Standards and Technology (NIST) has been Congressionally-funded to perform scientific foundation reviews of select forensic disciplines. These reviews are intended to establish what is well-known and well-supported empirically in a forensic field as well as where gaps may exist that need further study. DNA mixture interpretation was selected as the initial NIST scientific foundation review given the existence of abundant literature and a need expressed by members of the community. Multiple interlaboratory studies have noted variability among accredited laboratories using validated approaches to the same DNA mixture data.

DNA mixture interpretation has become more challenging in recent years due to several factors including submission of more touch evidence samples to aid investigations and generation of more sensitive DNA test results with new STR typing kits. This sensitivity enables recovery of DNA results from low quantities of biological material. However, the polymerase chain reaction (PCR) signal amplification process, which provides the DNA test sensitivity, also introduces artifacts into the resulting data including stutter products as well as allele drop-out, allele drop-in, and heterozygote imbalance that occur due to stochastic effects. These artifacts combined with allele sharing among contributors make separating the contributing genotypes difficult and therefore influence uncertainty related to both the interpretation process and associating potential contributors with the mixture. Probabilistic genotyping software programs are being implemented in many laboratories to aid evaluation of low-template DNA results either from single-source samples or trace components of mixtures.

For the past year (since September 2017), a NIST review team has been studying issues around DNA mixture interpretation. An important goal of this project is to identify, consolidate, and share core principles and supporting publications with the community to encourage deeper learning and understanding of DNA mixture interpretation. More than 500 articles related to DNA mixture interpretation have been gathered and are being examined to better understand capabilities and limitations as reflected in the scientific literature. An external DNA Mixture Resource Group, composed of 13 experienced practitioners, has provided valuable input and feedback to the NIST team on a regular basis.

INTRODUCTION

One of the biggest challenges faced today by forensic DNA laboratories is the interpretation of mixtures. Dr. Chantal Frégeau from the Royal Canadian Mounted Police offered her perspective in a February 2018 post [1]: “From a Biology/DNA discipline perspective, the highly sensitive STR [short tandem repeat] kits and capillary electrophoresis-based detection instruments currently used for forensic DNA typing analysis very often generate complex mixtures from ‘touch DNA’ exhibits brought in by the investigators. **The biggest challenge remains the interpretation of those complex mixtures and the determination of the relevance of a contributor’s DNA profile derived from an exhibit to the crime that has been committed**. Probabilistic software can assist with the interpretation of complex mixtures but determining how the genotypes were deposited remains challenging (relevance to the crime)” (emphasis added). Professor Bruce Budowle from the University of North Texas Health Science Center adds in this same post [1]: “Resources, education and training. Most of the issues we are facing seem to be related to these needs.”

Variation in mixture interpretation results across DNA laboratories have been reported with interlaboratory studies conducted by the National Institute of Standards and Technology (NIST) in 2005 and 2013 [2]. An article published in 2014 by a consortium of European laboratories points out that part of the challenge with DNA mixture interpretation lies in the fact that laboratories are operating using different rules. Loudres Prieto from the University Institute of Research in Forensic Sciences in Madrid and her co-authors write [3]: "There has been very little work published on the variation of reporting practices of mixtures between laboratories, but it has been previously demonstrated that there is little consistency. This is because **there is no current uniformity of practice, so different laboratories will operate using different rules**. The interpretation of mixtures is not solely a matter of using some software to provide 'an answer.'…" These authors express hope for a better future when structured training is implemented: "We show that **by introducing a structured training [program]**, it is possible to demonstrate, for the first time, that a high degree of standardization, leading to **uniformity of results, can be achieved by participating laboratories**" (emphasis added).

In September 2017, NIST initiated a scientific foundation review of DNA mixture interpretation to help define and compile what is known and foundational in the field [4]. A scientific foundation review is a study that seeks to identify and evaluate the body of scientific data and information that underlies a forensic method or practice. A scientific foundation review can (1) identify methods or aspects of methods that are built upon a solid scientific foundation in order to increase trust in those methods, (2) describe areas that could benefit from strengthening so that strategic focus can inform future research efforts, and (3) assist forensic practitioners and their stakeholders to develop a shared understanding of core principles with a desired outcome of (a) reducing variability in test results produced by different laboratories and (b) improving understanding of what results mean.

METHODS

Scientific Foundation Reviews: In recent years, several scientific advisory bodies[1] expressed the need for scientific foundation reviews of forensic disciplines to document and consolidate information supporting the technical soundness of methods used in forensic analysis and identify gaps where they exist. These groups identified NIST as an appropriate agency to perform the studies. In fiscal year 2018, Congress appropriated funds to NIST for this purpose, and NIST has begun reviews covering DNA mixture interpretation and bitemark analysis. In addition to providing insights into these specific disciplines, the initial reviews are serving as pilot studies for future efforts of this type.

In conducting a scientific foundation study, the NIST review team asks: "What empirical data exist to support the methods that forensic science practitioners use to identify and characterize evidence and associate it with people, places, and things from past events?" To answer this question, available data are collected and evaluated to determine whether the scientific approaches, methods, and practices (i.e., application of methods) for that discipline are well-supported and suitable for use (i.e., "fit-for-purpose").

NIST scientific foundation reviews are conducted by: (1) collecting and evaluating the peer-reviewed literature, (2) assessing available data from interlaboratory studies, proficiency tests, and laboratory validation studies, and (3) exploring other available information including position statements and non-peer reviewed literature.

The approach NIST is taking to the review of forensic science literature builds upon previous efforts and experiences [8]. These activities, which have often been conducted independent of other on-going or previous efforts, include literature reviews, input from advisory groups, and workshops. Many of these previous efforts have been prospective (i.e., looking to where the field needs to go) rather than introspective (i.e., reflecting on the foundations and support that exist for specific methods). An important goal of the NIST scientific foundation reviews is to consider, compile, and integrate information from previous efforts.

[1] The National Research Council of the National Academies of Science (2009) [5], the National Commission on Forensic Science (2016) [6], and the President's Council of Advisors on Science and Technology (2016) [7].

Background on NIST: NIST is a scientific research agency that is part of the U.S. government's Department of Commerce with a mission to promote U.S. innovation and industrial competitiveness by advancing measurement science, standards, and technology in ways that enhance economic security and improve our quality of life [9]. NIST has been involved in activities to strengthen forensic science since the 1920s, when physicist Wilmer Souder conducted precision measurements to assist hundreds of investigations involving handwriting, typewriting, and ballistic examinations [10]. During much of the past century, NIST research has advanced and strengthened the measurements and technologies underpinning forensic analysis of DNA, fingerprints, firearms and toolmarks, and digital evidence.

Review Team and Resource Group: The authors of this article comprise a six-member team at NIST with expertise in research, forensic DNA literature, statistics, human factors, casework management (the retired director of Forensic Science Ireland was hired on contract to assist with this project), and scientific writing and communication. A 13-member Resource Group composed of 9 experienced practitioners (3 Federal, 3 state, 2 local, 1 Canadian) and 4 academic researchers or consultants, who are identified by name in the acknowledgments, has provided regular input and acted as a sounding board during this study.

RESULTS & DISCUSSION

Foundational Literature Bibliography: A list of over 500 articles related to DNA mixture interpretation has been compiled to help create a foundational bibliography for the field. These articles were identified through on-line searches using PubMed [11] as well as examining references cited in applicable articles. Relevant peer-reviewed journals were scoured including *Forensic Science International: Genetics*, *International Journal of Legal Medicine*, and the *Journal of Forensic Sciences*. From these publications, underlying principles are being identified along with expressed capabilities and limitations of methods used. The creation of a comprehensive, curated reference list is expected to be a valuable output of this review.

Ten Key Articles: As a precursor to the larger reference list, which is still in development, a set of ten key articles has been identified along with some of the important principles being conveyed and questions that might be asked in thinking about how DNA mixture interpretation is being approached. Of course, additional articles are helpful as well. These "top" ten articles and principles to consider in question form include:

1. Are we **addressing the right question(s)** with our results? [12]
2. Are we aware of possible **stochastic effects**? [13]
3. Are we able to **deconvolute the mixture into component genotypes**? [14]
4. Are we recognizing **peaks in stutter positions as potential minor alleles**? [15]
5. Are we **aware of variation** in how others may approach a mixture? [2]
6. Are we performing **validation studies to estimate drop-out and drop-in probabilities** with known samples? [16]
7. Are we assessing **performance with potential non-contributors**? [17]
8. Are we reporting results with **clear propositions and limited significant figures**? [18]
9. Are we **disclosing assumptions made** and contextual information used? [19]
10. Are we **thinking carefully about the case data and context** and not just feeding information into a computer program? [20]

Identifying and appreciating important principles can lead to a greater uniformity of practice across the community as forensic DNA analysts operate using the same rules.

CONCLUSION

A report describing findings from this initial NIST scientific foundation review of DNA mixture interpretation is being prepared with plans to release it first in draft form to seek feedback from the forensic science community and stakeholders such as judges, attorneys, and members of the public that utilize DNA results to make decisions. A NIST website will house copies of the initial draft and eventual final report as well as supplemental documents to assist in understanding the information gathered and evaluated.

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REFERENCES

1. https://www.ishinews.com/biggest-challenge-forensics-laboratories-face-today/
2. Butler, J.M., Kline, M.C. and Coble, M.D. (2018) NIST interlaboratory studies involving DNA mixtures (MIX05 and MIX13): variation observed and lessons learned. *Forensic Sci. Int. Genet.* 37: 81-94.
3. Prieto, L., Haned, H., Mosquera, A., Crespillo, M., Aleman, M., Aler, M., Alvarez, F., Baeza-Richer, C., Dominguez, A., Doutremepuich, C., Farfán, M.J., Fenger-Grøn, M., García-Ganivet, J.M., González-Moya, E., Hombreiro, L., Lareu, M.V., Martínez-Jarreta, B., Merigioli, S., Milans Del Bosch, P., Morling, N., Muñoz-Nieto, M., Ortega-González, E., Pedrosa, S., Pérez, R., Solís, C., Yurrebaso, I. and Gill, P. (2014) Euroforgen-NoE collaborative exercise on LRmix to demonstrate standardization of the interpretation of complex DNA profiles. *Forensic Sci. Int. Genet.* 9: 47-54.
4. https://www.nist.gov/news-events/news/2017/10/nist-assess-reliability-forensic-methods-analyzing-dna-mixtures
5. National Research Council, Committee on Identifying the Needs of the Forensic Science Community (2009) *Strengthening Forensic Science in the United States: A Path Forward*. The National Academies Press: Washington DC.
6. National Commission on Forensic Science, Recommendation to the Attorney General (approved September 12, 2016), "Technical Merit Evaluation of Forensic Science Method and Practices"; available at https://www.justice.gov/archives/ncfs/page/file/905541/download
7. President's Council of Advisors on Science and Technology (PCAST) Report to the President: *Forensic Science in Criminal Courts: Ensuring Scientific Validity of Feature-Comparison Methods* (released September 20, 2016): https://obamawhitehouse.archives.gov/sites/default/files/microsites/ostp/PCAST/pcast_forensic_science_report_final.pdf
8. NIST Forensic Science Foundation Reviews: https://doi.org/10.6028/NIST.IR.8225-draft; see also https://www.nist.gov/news-events/news/2018/09/nist-details-plans-reviewing-scientific-foundations-forensic-methods
9. https://www.nist.gov/about-nist/our-organization/mission-vision-values
10. Press, R. (2017) Who was Detective X? https://www.nist.gov/featured-stories/who-was-detective-x
11. https://www.ncbi.nlm.nih.gov/pubmed
12. Taroni, F., Biedermann, A., Vuille, J., and Morling, N. (2013). Whose DNA is this? How relevant a question? (a note for forensic scientists). *Forensic Sci. Int. Genet*. 7: 467-470.
13. Walsh, P.S., Erlich, H.A. and Higuchi, R. (1992) Preferential PCR amplification of alleles: mechanisms and solutions. *PCR Methods Appl.* 1(4): 241-250.
14. Clayton, T.M., Whitaker, J.P., Sparkes, R. and Gill, P. (1998) Analysis and interpretation of mixed forensic stains using DNA STR profiling. *Forensic Sci. Int.* 91(1): 55-70.
15. Gill, P., Brenner, C.H., Buckleton, J.S., Carracedo, A., Krawczak, M., Mayr, W.R., Morling, N., Prinz, M., Schneider, P.M. and Weir, B.S. (2006) DNA Commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures. *Forensic Sci. Int.* 160: 90-101.
16. Gill, P., Gusmao, L., Haned, H., Mayr, W.R., Morling, N., Parson, W., Prieto, L., Prinz, M., Schneider, H., Schneider, P.M. and Weir, B.S. (2012) DNA commission of the International Society of Forensic Genetics: Recommendations on the evaluation of STR typing results that may include drop-out and/or drop-in using probabilistic methods. *Forensic Sci. Int. Genet.* 6(6): 679-688.
17. Gill, P. and Haned, H. (2013) A new methodological framework to interpret complex DNA profiles using likelihood ratios. *Forensic Sci. Int. Genet.* 7(2): 251-263.
18. Steele, C.D. and Balding, D.J. (2014) Statistical evaluation of forensic DNA profile evidence. *Annu. Rev. Stat. Appl.* 1: 361-384.
19. Gill, P., Hicks, T., Butler, J.M., Connolly, E., Gusmão, L., Kokshoorn, B., Morling, N., van Oorschot, R.A.H., Parson, W., Prinz, M., Schneider, P.M., Sijen, T. and Taylor, D. (2018) DNA Commission of the International Society for Forensic Genetics: Assessing the value of forensic biological evidence – guidelines highlighting the importance of propositions. Part I: Evaluations of DNA profiling comparisons given (sub-) source propositions. *Forensic Sci. Int. Genet.* 36: 189-202.
20. Gill, P., Haned, H., Bleka, O., Hansson, O., Dorum, G. and Egeland, T. (2015) Genotyping and interpretation of STR-DNA: Low-template, mixtures and database matches-Twenty years of research and development. *Forensic Sci. Int. Genet.* 18: 100-117.