

Final Report on
Review of Mixture Interpretation in Selected Casework of
the
DNA Section
of the
Forensic Science Laboratory Division (FSL),
Department of Forensic Sciences (DFS),
District of Columbia

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Preface

The Department of Forensic Sciences (DFS) was created through the “Department of Forensic Sciences Act of 2011” by the Council of the District of Columbia. Operations as an agency commenced on October 1, 2012. The DFS is comprised of several divisions, including the Forensic Science Laboratory Division (FSL), the Public Health Laboratory Division, and the Crime Scene Sciences Division, all located along with the Office of the Chief Medical Examiner (OCME) in a Consolidated Forensic Laboratory at 401 E Street S.W. Washington, DC.

The DFS provides various services to a number of agencies, including the Metropolitan Police Department, the Office of the Chief Medical Examiner, the Office of the Attorney General, the Department of Health, the Fire and Emergency Medical Services Department, the United States Attorney’s Office for the District of Columbia, and other law enforcement or investigative agencies.

Over the past few years the United States Attorney’s Office for the District of Columbia (USAO) has requested the assistance of various experts, outside of the DFS and its predecessor laboratory, in preparation for admissibility hearings and trial testimony, some relating to forensic DNA evidence. Such experts have included the authors of this Report.

In May 2014, the USAO requested the assistance of Dr. Bruce Budowle in providing additional statistical calculations, not performed by DFS, relating to a DNA mixture profile from a particular evidence item in preparation for an upcoming trial (U.S. v. Tavon Barber, 2013-CF1-011157). During review of the DNA results in that case analyzed by the FSL of the DFS, in preparation of his trial testimony, Dr. Budowle identified several concerns regarding interpretation of the DNA evidence by DFS, specifically regarding selection of interpretable genetic markers for statistical calculations and DNA mixture deconvolution. Dr. Budowle prepared his own independent analysis and testified in the trial. After this review, a USAO representative attended a DFS Scientific Advisory Board meeting on October 7, 2014 to present the concerns raised by Dr. Budowle about mixture interpretation at the DFS.¹

At the advice of Dr. Budowle (based on his concerns in the review of the Barber case), the USAO began reviewing pending cases in which DFS had issued reports with Combined Probability of Inclusion (CPI) statistics. During this time frame, the USAO, with the assistance of Dr. Budowle, communicated telephonically with DFS management including the DNA technical leader and two members of the DFS Scientific Advisory Board and later delivered a telephonic PowerPoint presentation illustrating the issues he and the USAO had identified regarding DFS mixture interpretation practices. Dr. Budowle reviewed a number of additional pending cases, at the request of the USAO, and identified additional issues regarding mixture interpretation, including CPI statistics and mixture deconvolution, and recommended a more comprehensive review.

¹ Prior to the formation of the "Panel" issuing this Report, DFS performed a “non-exhaustive” review of 27 cases involving DNA evidence. Seven involved DNA mixtures, 3 of which included DNA mixture statistics. Of these 3 cases, 2 had CPI calculations one of which was modified by DFS after its review. This limited review was deemed insufficient by Dr. Budowle which then led to a more comprehensive review by USAO.

In response, the USAO retained a panel of experts consisting of Dr. Bruce Budowle, Dr. Frederick R Bieber, and Ms. Lisa Brewer (hereinafter referred to as “the Panel”) to perform review of all pending cases and others which involved prior convictions, in which DFS had issued a DNA report with statistical calculations.²

In review of such cases and DFS reports, the USAO instructed the Panel to identify any instances in which, in their opinion, the interpretation of DNA evidence or the accompanying statistical analysis was questionable, but not those which could be attributed to acceptable variation of DNA interpretation within the relevant scientific community. However, the Panel did take note of inconsistent practices by DFS analysts that might impact the interpretation of DNA results and statistical analyses.

Lastly, the Panel was asked to assess what measures need to be implemented before the USAO can resume using DFS for DNA testing. To accomplish that assessment, the Panel, with the permission of the D.C. Mayor’s Office, conducted a two-day site-visit of DFS. The USAO also asked the Panel to make recommendations about the need for additional training on DNA mixture analysis, interpretation, and reporting, as well as the formal technical review process and to offer to develop and deliver a practical competency assessment to help assure that the above protocols, procedures, review processes, and continuing education meet the needs of the DFS in order to attain high quality DNA typing results. This Report summarizes the results of the Panel’s work and findings, to date, and addresses some of the needs and recommendations for the future.

² Past convictions and pending cases involving DFS mixture interpretation were selected for review by the USAO and then screened initially by Dr. Bruce Budowle. If issues of concern were identified, the case materials were forwarded to the entire Panel for review. After the USAO decided to send case work to the Verdugo Regional Crime Laboratory in California, Lisa Brewer no longer served on the Panel in order to avoid a potential conflict of interest.

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Abstract

Scope and Goals of the Project (December, 2014 to the present)

The work of the Panel involved review of selected DNA casework performed in the DNA section of the Forensic Science Laboratory Division (FSL), Department of Forensic Sciences (DFS), District of Columbia (DC). This project was performed at the request of the U.S. Attorney's Office for the District of Columbia (USAO) in the aftermath of concerns raised by Dr. Budowle regarding interpretation and statistical analysis of forensic DNA evidence in specific cases.

The goals of the Panel included general review of interpretation and statistical evaluation of forensic DNA mixtures in cases identified and selected by the USAO. The purpose was to identify specific issues of concern in the selected cases analyzed by the DFS and, through the on-site visit and interviews with members of the FSL staff, to identify ways to improve laboratory performance through additional education and training of DNA analysts, standardization of technical reviews, and to plan for the future needs.

The Panel was asked to review prior convictions and pending cases (N=68 as of the date of this Report) involving forensic DNA mixtures with specific focus on selection of alleles or loci used for generating statistical estimates to determine whether they comport with acceptable practices. The Panel evaluated these cases following the DFS standard operating protocols (SOPs) and relied on the analytical and stochastic thresholds established by DFS.

The Panel identified several thematic concerns from an initial review of selected DFS cases as well as a number of problems in interpretation of DNA mixtures in many of the cases selected by the USAO for review. The Panel's thematic concerns and specific findings were forwarded directly to the USAO. Several examples are included herein (see Appendix).

Based on an on-site visit, review of DFS documents and interviews with members of the DNA section and laboratory management, the Panel has identified necessary changes to the evaluation of forensic DNA mixtures at the DFS and recommended initiatives for improvement. The Panel also identified needs for additional training, education, and proficiency testing of members of the DNA unit and recommends improvements in the technical review procedures prior to issuing final reports along with better formalized communication with laboratory management. These changes and initiatives will be needed considering the anticipated increased demand for forensic DNA services and need for reduction of existing case backlogs at the DFS.

The Executive Summary that follows addresses the findings and opinions of the Panel, and its recommendations for the USAO and the DFS to consider.

Executive Summary

1) At the request of the USAO, the Panel reviewed selected forensic casework involving DNA mixtures that were analyzed by the FSL of the DFS. In addition, the Panel performed an on-site review of DNA operations at the FSL of DFS for the purpose of assessment of general operations and laboratory protocols as they relate to interpretation of forensic DNA profiles. The USAO also asked the Panel to address whether any additional DNA interpretation training is warranted for DNA analysts at the DFS. As requested, the Panel communicated its opinions and concerns about selected cases directly to the USAO. Work completed, in progress, and recommendations for the near future are summarized below.

2) With regard to the thematic issues raised and concerns about the initial set of cases reviewed, the Panel respectfully disagrees with DFS management's response that its practices related to DNA mixture interpretation are appropriate and within the range of generally acceptable practices. Several examples from cases reviewed by the Panel are provided (see Appendix) to illustrate what the Panel considers fundamental problems with the interpretation of forensic DNA mixtures in certain evidence in specified casework.

3) Problems identified by the Panel in specific DFS cases included:

- a) inappropriate use of the combined probability of inclusion statistical approach (CPI) in mixtures by inclusion of loci where allele drop out was highly probable;
- b) inappropriate use of the CPI in mixtures by including individuals whose known alleles were not present, at those loci, in the evidence samples;
- c) inappropriate calculation of two separate CPIs for the same forensic DNA mixture profile;
- d) not using established stochastic thresholds to assess potential allele drop out, and
- e) inconsistencies and deficiencies in the technical review process of the DNA analysis pipeline.

4) A comprehensive review of mixture cases should be performed by DFS. The Panel had concerns, in some cases, regarding the DFS methods of mixture deconvolution and also identified instances in which cognitive (interpretation) bias in mixture interpretation may have been present. The Panel noted inconsistent practices regarding subtraction of profiles of known victims from DNA mixtures taken from intimate evidentiary samples. Such variation, along with the findings noted above, led the Panel to conclude that both the analysis and the technical review steps in the DFS DNA analysis pipeline require improvement.

5) The Panel performed a 2-day site visit of the FSL at the DFS and interviewed DNA analysts in the DNA section. The Panel found the FSL facilities to be outstanding providing every opportunity for success. The Panel was favorably impressed with the dedication of the staff of the DNA unit, yet was concerned when informed by most of those interviewed that they had not been apprised of case-specific concerns previously raised by the Panel regarding interpretation and statistical evaluation of specific evidentiary items or cases. The Panel found that the DFS responses did not address the Panel's thematic concerns about DNA mixture interpretation and

reporting. Without improved communication and open discussion of case-specific concerns the staff members' ability to address deficiencies and enhance performance is hampered.

6) The technical review component of the DNA pipeline needs substantial improvement with concomitant better documentation. The processes in place at the time of the visit were not sufficiently stringent to identify interpretation errors and challenges and to reduce substantial variation in DNA mixture interpretation and statistical analysis among the analysts.

7) The Panel recommends additional training on DNA mixture interpretation for the DNA analysts and technical leader at DFS prior to performing additional casework involving forensic mixtures. This training should include evaluation of DNA mixtures, technical review processes, validation strategies, and other topics focused on minimizing potential for cognitive (interpretation) bias. After additional training and demonstration of competency, assessment of performance should be monitored if the USAO returns DNA casework to DFS. Only with such an effort can the DFS analysts achieve the goal of being highest quality service providers which each and every one of them professed as his/her desire and commitment.

Review of Selected DNA Casework

At the request of the USAO, the Panel reviewed selected convictions and pending cases (N=68 as of the date of this Report) primarily regarding the alleles or loci used for generating the reported statistic(s) to determine whether they comport with correct practices within the general scope of the current DFS SOP in effect at the time the DFS final reports were generated.

The Panel members are well aware of the methods used by forensic DNA analysts to evaluate and interpret forensic DNA mixture evidence. Such protocols involve applying similar methods to those used to evaluate single source DNA profiles with the additional special focus on factors such as PCR artifacts, PCR stutter, allele and locus drop-out and drop-in, allele stacking or sharing, DNA degradation, etc. Mixture evidence is far more common and complex today than it was at the inception of forensic DNA typing. Statistical evaluation of such DNA mixture evidence requires careful interpretation of results and application of appropriate statistical tools to perform calculations which estimate random match probabilities, the so-called Combined Probability of Exclusion/Inclusion (herein after referred to as the CPI), and likelihood ratios comparing the probabilities of DNA evidence under competing hypotheses.

On January 29, 2015 the DFS issued a memo in response to the Panel's concerns based on its initial review of selected DNA mixture casework. In this memo the DFS response was

"All of the reported issues fall under the general category concerning the DNA mixture interpretation guidelines within the Unit. On January 27, 2015, the reported issues and related cases were reviewed in depth by DFS personnel. The general finding of the review were ultimately seen as a difference of opinion between experts in regards to all five of the noted issues. The arguments and criticisms raised in the USAO report were not found to be persuasive. In all cases, it was seen that the Unit personnel issuing the reports adhered to the Unit's DNA mixture interpretation guidelines that were in place at the time the work was performed on the cases. "

The Panel respectfully disagrees with the response by the DFS. Indeed, many of the concerns raised by the Panel relate to basic and fundamental aspects of a mixture interpretation process and the use of the CPI. The Panel appreciates that the CPI is considered to be an acceptable statistical approach to evaluation of forensic DNA mixtures. It also is well-established that the CPI cannot be applied to individual loci in situations where allelic drop out is either evident or probable. The Panel is aware that some of the DFS analysts have received some training on interpretation of DNA mixtures during the summer of 2014, prior to the time that concerns were raised by USAO. The Panel reviewed this particular training material which noted explicitly that the CPI cannot be applied in situations where there is a high probability of allele drop-out. The Panel agrees with this position on allele drop-out and application of the CPI. This same accepted principle was used by the Panel in its interpretation of CPI calculations performed by the DFS in selected cases that were reviewed.

Moreover, the approaches for using CPI are well-documented and described in the literature and in a textbook written by Dr. John M. Butler (“Advanced Topics in Forensic DNA Typing: Interpretation”, Academic Press/Elsevier, 2014). Textbooks assemble common knowledge and practices and thus can convey the community wide understanding of what is considered to be generally acceptable, within the wide range of acceptable practices.

The passages below, taken directly from Dr. Butler's book, provide some examples of what are considered acceptable standards for calculation of the CPI when analyzing forensic DNA mixture evidence.

Page 321 - “CPI is based on the evidence only. Selecting different loci for comparison purposes, something often referred to as “suspect-driven CPI” is inappropriate since decisions on which loci are suitable for comparison should be made prior to doing a comparison to reference sample(s).”

Page 335 – “CPI can be a valid statistical representation of DNA mixture data provided that there are no missing alleles.”

Page 335 – “Keep in mind also that a higher number of contributors dilutes out the amount of DNA for each contributor, which leads to more stochastic effects and the possibility of allele dropout and therefore less certainty in the overall interpretation.”

Page 336 – “Urban Legend #6: If all peaks at a locus are above the established stochastic threshold, then the locus is safe to use. Allele stacking is a possibility (see Figure 6.6), especially with less polymorphic STR loci, such as TPOX and D5S818. Therefore, having for example TPOX alleles 8 and 11 above an established stochastic threshold (a situation that could occur due to allele stacking) does not mean that allele drop-out did not occur with one of the contributors to this mixture. This urban legend relates to Urban Legend #1 regarding the number of potential contributors.”

Page 336 – “Urban Legend #8: Suspect-driven CPI (where the comparison of each suspect results in a different statistical result) is fine. The CPI statistic is calculated from the evidence profile and should not vary based on the reference profile.”

Page 549 – “There are several requirements to consider before the RMNE approach can be appropriately used: (1) the individuals in the mixture are unrelated, (2) the individuals are from the same population group, and (3) all of the alleles in the profile are present (no drop-out), which is presumed by having all alleles at a locus possess peak heights above the stochastic threshold. Only loci where all of the alleles are present above the stochastic threshold should be used in the CPI statistical calculation. If there is any indication that data may be missing at the examined locus, perhaps due to the presence of allele peaks below the stochastic threshold that may raise the possibility of a missing sister allele, then anyone could technically be included in the mixture and the statistical weight of the locus would have a probability of 1.”

These textbook passages on some of the fundamental principles of the application of the CPI, and its limitations, are consistent with the opinions of the Panel and not with the manner that DFS selected alleles and loci utilized in CPI calculations in selected casework reviewed at the request of the USAO. The Panel is well aware of the variety of practices in the forensic DNA community and reiterates that it was asked by the USAO to identify instances in which the interpretation of DNA evidence or the accompanying statistical analysis was questionable, but

not those which could be attributed to acceptable variation of DNA interpretation within the relevant scientific community.

Accordingly, the Panel identified a number of instances of flawed interpretation of DNA mixtures which were summarized previously and communicated to the USAO. Several examples are described herein (see Appendix) to help illustrate the Panel's findings that some of the interpretive practices at DFS were not supported by generally accepted scientific principles and are beyond what the Panel considers acceptable variation in the relevant scientific community.

Referring again to the DFS Memo of January 29, 2015, DFS is implementing a new SOP,

"Specifically, the proposed changes to the mixture interpretation protocols will address all of the issues raised by the scientific panel appointed by the USAO and by the DFS' Scientific Advisory Board. These protocols will include the documented justification of mixture identification, mixture deconvolution, and the determination of the number of potential contributors to a DNA mixture. The statistic calculation protocols will address the statistical inclusion or exclusion of individuals within a DNA mixture based on Combined Probability of Inclusion (CPI) methodologies, and when CPI should be applied as a calculation."

Action Item: The Panel recommends review of all previously reported DFS cases involving DNA mixtures to determine whether the new SOP addresses the thematic concerns identified by the Panel. In addition, the Panel recommends assessment of performance for a defined time period if the USAO returns DNA casework to DFS.

On-site DFS Visit (February 19-20, 2015)

The USAO requested the Panel to review DNA operations at the FSL of DFS for the purpose of assessment of general DNA Unit operations and laboratory protocols as they relate to interpretation of forensic DNA profiles. The USAO also asked the Panel to recommend whether any additional DNA interpretation training is warranted for DNA analysts at the DFS.

A 2-day on-site visit of DFS was conducted on February 19 and 20, 2015. The Panel conducted interviews with members of the Forensic Biology Unit and some members of the managerial staff at DFS. The DFS Director, General Counsel, and Quality Assurance Manager were not present during the 2-day visit and were interviewed subsequently in a follow-up telephone conference on February 25, 2015.

The Panel, accordingly, reviewed the DNA pipeline in place at DFS which included:

1. an on-site visit for 2 days to review the updated protocols, equipment and validation studies, recent audits, and workflow pipelines, to include the internal case technical and administrative review procedures;
2. review of all DNA training materials and recent updates to laboratory protocols, bench worksheets and statistical worksheets; and
3. meetings with some of the key DFS personnel who perform and oversee DNA analyses to review background and level of experience with forensic DNA analyses.

The Panel requested specific materials for review, including:

1. DNA SOPs and work instructions, to include the newly implemented SOPs and the criteria for inclusion and exclusion,
2. summary of review process in general, including case review, technical review, and administrative review,
3. DNA and General Lab Documents,
4. past two internal and external audits,
5. corrective action policies,
6. records of problems and remediations,
7. DNA mixture validation studies,
8. Internal validations to determine detection thresholds and stochastic thresholds at all conditions utilized (e.g. 28 cycles, 29 cycles),
9. machine noise testing,
10. personnel files relating to:
 - a. university transcripts;
 - b. transcripts of testimony;
 - c. proficiency test scores;
 - d. training programs including in-house and continuing education;
 - e. corrective action pertaining to specific analysts;
 - f. qualifying exams; and
11. documentation relating to meetings of management with personnel regarding policy and guidance regarding DNA typing for the last 12 months.

Findings from the On-Site Visit of DFS

Interpretation and Statistical Analysis of DNA Mixtures

During the on-site visit the Panel met with most members of the DNA unit to discuss general issues surrounding the new SOP and DNA mixture interpretation. The goal was to try to understand the background and level of training of the DNA staff and to try to determine the rationale to explain why for example, in some of the cases reviewed by the Panel, a CPI statistic was applied using loci at which the allele(s) of individual(s) who were not excluded as possible contributors were not observed in the evidence profile itself (*The CPI estimates the portion of the relevant population(s) that could be potential contributors to a DNA mixture profile. If the same alleles present in a suspect's known sample are not seen in the mixture evidence, then the CPI cannot be used. Thus, if a CPI estimate were generated, it would have no relevance regarding the potential inclusion of that particular suspect*). For a locus to be included in a CPI there should not be convincing evidence or a high probability of allele or locus drop out. During the on-site interview the technical leader stated she did not recall such a practice at DFS, although the Panel identified such examples in the casework reviewed.

During the on-site visit, the Panel was informed that four upper management personnel (two of whom had forensic DNA analysis technical experience) were those who reviewed the thematic issues and specific cases of concern that had been initially identified by the Panel. The Panel was informed that these specific cases were not discussed with the Technical Leader or the DNA analysts assigned to the cases. There were no other materials available to the Panel for review other than the unsigned memo sent to USAO that conveyed upper management's position.

Despite a number of attempts by the Panel during the on-site visit to learn about the scientific bases of upper management's position and if there were any differences in opinion regarding the specific cases, those who were interviewed declined to engage in any discussion other than to state that the DFS position taken was an "agency position". Therefore, those at DFS who were familiar with specific concerns raised by the Panel provided little insight regarding the underlying scientific principles used to support the DFS position on the issues raised by the Panel. One point that was reiterated repeatedly was that a statistical estimate could vary, for example, between 1/2000 and 1/1000 and such differences are minor or meaningless; thus there should be little concern if a locus or two used in calculations were questioned by the Panel or the USAO. Such a position is not sustainable as some of the statistical estimates, when calculated in accord with the Panel's recommendation, changed by several orders of magnitude (even from 1 in millions to 1 in tens) or could not be calculated at all.

It is the opinion of the Panel that it is not appropriate to justify use of a particular protocol or interpretive practice based on differences in numerical statistical estimates. The Panel agrees that statistical estimates can vary somewhat, that some of the differences might not be significantly different from one another and that the confidence intervals of different estimates might overlap. That fact notwithstanding, it is concerning if interpretive errors are not identified prior to issuing a final report and, if such errors do occur, that they might not be addressed simply because the degree of variation in a statistical calculation may be deemed nominal.

At the time of the Panel's site visit, DFS was in the process of implementing new SOP for DNA mixture interpretation. The DFS memo of January 29, stated,

"Specifically, the proposed changes to the mixture interpretation protocols will address all of the issues raised by the scientific panel appointed by the USAO and by the DFS' Scientific Advisory Board. These protocols will include the documented justification of mixture identification, mixture deconvolution, and the determination of the number of potential contributors to a DNA mixture. The statistic calculation protocols will address the statistical inclusion or exclusion of individuals within a DNA mixture based on Combined Probability of Inclusion (CPI) methodologies, and when CPI should be applied as a calculation."

Action Item: Additional training on DNA mixture interpretation should be offered along with competence assessment of DNA analysts (and relevant supervisors) prior to performing any additional casework. A more formalized root cause analysis process should be implemented at DFS to address the issues noted above. Once additional training and competency assessment has been successful there should be an interim period of review of cases going forward under the recently modified SOP.

Technical Review Process

The technical review process in the FSL DNA analysis pipeline appears to the Panel to be woefully inadequate. The Panel noted inconsistencies among analysts in how they selected loci for inclusion in statistical calculations as well as errors in reports and case files which escaped notice in technical or administrative review. For example, the Panel was informed that use of two different CPIs for the same mixture profile was due to practices of two former DNA analysts and, that, because they are no longer employed, this practice of calculating two CPIs for a single mixture is no longer an issue. Yet, the current staff (including the technical leader) served as technical reviewers in such cases (for example, see **US v. Roble 2013-CF1-6095, DFS Lab # M130206-1**). The Panel believes that both the SOP and technical review should have identified this issue prior to issuing a report.

Action Item: While the new SOP contains a helpful documentation worksheet, the technical review process did not appear to the Panel to be addressed adequately. As some of the DNA technical reviews were performed by current DNA staff members, additional training and education on performance and documentation of a thorough technical review are needed. Based on review of casework and discussions during the on-site visit, it is the opinion of the Panel that the current DNA technical leader deserves more guidance and support from DFS management in order to address issues of concern to the Panel and to successfully lead the DNA Unit.

Potential for Interpretation Bias

Interpretation bias, a form of cognitive bias, is often an inherent, sometimes subconscious, human tendency to interpret unclear or vague results into a positive or negative outcome.

Processes should be implemented in forensic laboratories to recognize and reduce interpretation bias. One such process involves the technical review component of the analysis pipeline. The Panel found several examples involving DNA mixture interpretation problems that are consistent with the possibility of such bias. In **US v Jeffrey Neal (2014-CF1-010507, DFS Lab # M140492)**, the original DFS report for item 26c1.1_26c1.2 stated “The major male contributor is consistent with the DNA profile obtained from the known sample from Jeffrey Neal (Item 76).” The Panel disagreed with the interpretation by DFS. To assess whether interpretation bias might have factored into the original interpretation by DFS an electropherogram from this case (item 26c1.1_26c1.2) was redacted and shown to a DNA analyst and the technical leader during the on-site interviews. The DNA analyst and the technical leader were each asked to interpret the profile under both the new SOP and the interpretation guidelines employed when this case was analyzed. The Panel notes that the analyst was the original technical reviewer of the electropherogram. To reduce the chance for interpretation bias the analyst and the technical leader were not shown the reference profile of the suspect when asked to interpret the mixture profile in determination of the types of the major contributor (as this is the proper practice). Both opined that the profile of the suspect would be excluded as the major contributor of the profile (an interpretation consistent with that of the Panel but discrepant with the actual final laboratory report). Both stated that they would reach this same interpretation whether they used the previous or currently enacted SOPs for mixture interpretation. It is noteworthy that DFS upper management reviewed this same case and supported the original interpretation.

This observation (and others) supports the view that DNA analysts need to be aware of the possibility of and try to avoid interpretation bias in the analysis and interpretation of DNA mixture evidence as the technical review process alone might not be sufficient at identifying and correcting all analytical and interpretation errors.

Action item: The technical review process should be formalized to address the potential of interpretation bias in forensic laboratories. Casework should be reviewed to identify and address any instances in which such bias affected interpretation of results. Training and continuing education of staff should include lectures on cognitive bias, how it affects interpretation, and tell-tale signs to identify when it may arise.

Analytical Threshold Validation

The Panel was informed that DFS has implemented a new DNA SOP (for interpretation) that contains a requirement of more documentation during the analytical phase of the DNA analysis pipeline. The new SOP makes reference to different analytical thresholds (ATs) for DNA results above and below 1000 RFUs. The Panel expressed concern that the new derived AT thresholds may be inappropriate as different types of samples were used to determine the AT values. The AT thresholds above 1000 RFU were derived from samples amplified with ideal target quantities of DNA while the AT thresholds below 1000 RFU were based on data derived from 29 negative samples. The Panel notes that using different data sets for AT thresholds – a lower set for less than 1000 rfu data and a higher set for above 1000 rfu - is not appropriately derived from the DFS validation study, as baseline "noise" from samples that contain DNA is higher than from

samples that do not contain DNA. Samples with low and ideal target quantities of DNA inherently will have more "noise" than "negative" samples. Accordingly, higher AT thresholds are appropriate for such samples.

Action Item: The Panel recommends additional input for performing, interpreting, and applying validation data is required for upper management and staff.

New DFS Policy on Minimum # of Loci

The Panel was informed that DFS has instituted a new policy which requires a minimum of six loci to be interpretable to report a statistic for a DNA mixture deemed to be composed of three or more individuals. The rationale for this decision was not clear to the Panel, as there could be instances for which DNA results on less than six loci may be probative for either the defense or the government. Therefore, the Panel, through the USAO, requested additional information on the rationale for selecting a six loci threshold. The Panel was informed through this communication from DFS that this decision was based, in part, on CODIS upload requirements.

The Panel notes that the principal reasons that CODIS (either NDIS, SDIS or LDIS) selects a minimum locus threshold for uploads and searches is for managing the number of adventitious hits and subsequent downstream workloads. The DFS justification for reporting DNA mixture statistics, based on a potential upload to CODIS, does not consider the statistical power that can be present even with less than six loci and it does not consider how DNA evidence can support alternate hypotheses during litigation. The potential value of even limited evidence could be meaningful for both defense and prosecution.

Action Item: The Panel recommends that DFS fully engage its customers before implementing such a new policy.

29-Cycle PCR

In the cases reviewed by the Panel, DFS employed two different PCR protocols for DNA analysis, i.e., a 28 cycle protocol and a 29 cycle one. The former is used routinely and if a sample yields low DNA profile signal results and at the discretion of the analyst, the 29 cycle PCR protocol is employed. The different PCR cycles have different stochastic thresholds (STs) – the 28 cycle has a 200 rfu ST, and the 29 cycle has a 300 rfu ST.

The Panel noticed that with the 29 cycle protocol stochastic effects may be increasing compared with the 28 cycle protocol, and some interpretations of results appeared to be associated with cognitive (interpretation) bias (as described above in *US v Jeffrey Neal*). Initially, the Panel considered further review of DFS's validation studies regarding the 29 cycle protocol and whether the findings of these studies comport with the current SOP. As the new DNA SOP does not describe interpretation of DNA results generated from the 29 cycle protocol, the Panel

questioned whether the 29 cycle protocol was still being employed at DFS³. On April 14, 2015 the DFS informed the Panel, through the USAO, that this 29 cycle protocol was discontinued in June 2013. Given the potential of increased stochastic effects to affect reliability of results, cessation of the 29 cycle protocol should be investigated further. Elimination of the 29 cycle protocol is noteworthy as some of the cases identified by the Panel employed the 29 cycle methodology with interpretation problems. One of the cases described herein (**United States v. Breal Hicks, et al, 2013-CR-203 (RJL), DFS Lab # M130107-1**) is an example of the panel's findings of problematic interpretation which involves a 29 cycle-generated STR profile.

Action Item: Additional review will be needed of cases in which the 29 cycle protocol was used along with notification or clarification in adjudicated and pending cases in which it was utilized. Furthermore, quality assurance practices need to be formalized to include better communication to DFS customers about any material changes in protocols.

Post-audit telephonic conversation with DFS officials

During the telephonic discussion with top DFS officials on February 25, 2015, among the explanations offered for the Laboratory's responses on thematic issues raised by the Panel and with regard to the specific casework in question included the following three;

1) The DFS lab followed its own protocol, there is no absolute standard, and "other labs do it that way".

The Panel again refers DFS to the Butler text and to their own past and recent training material and reiterates its opinion that the thematic issues raised require corrective action.

2) Nowhere is it explicitly stated that DFS practices of concern identified by the Panel cannot be performed in the manner DFS has done it.

Again, while the Panel is aware of a variety of practices in the community, some of the approaches used by DFS are simply not sustainable, and,

3) A materiality criterion (i.e., whether someone was wrongly convicted as a result of the interpretations used by DFS).

The Panel finds that materiality does not address the specific thematic issues raised by the Panel. Materiality is the responsibility of those in the legal system and can change throughout the course of an investigation, legal arbitration, or trial. Laboratories often do not necessarily have access to materiality, and in a case in which materiality is of issue resolution may take years.

³ Deduction of termination of the 29 cycle protocol was based on the lack of description for interpretation in the newly instituted SOP and from a telephone communication between Dr. Budowle and a former employee.

As mentioned earlier, the Panel had been informed by those most knowledgeable and intimately involved with the DNA casework (i.e., the technical leader and DNA analysts) that they were not involved in any attempt at a root cause analysis of the issues raised initially by the Panel. Such analysis and corrective actions, when needed, require enhanced communication with those involved in DNA typing process.

Action Item: A full root cause analysis is recommended so that the issues raised by the Panel can be carefully and systematically addressed.

Summary of Recommendations to Consider

1) Additional Training and qualifying exams for DNA analysts. The Panel recommends additional training on DNA mixture interpretation along with competence assessment of DNA analysts (and relevant supervisors) prior to performing any additional casework involving forensic mixtures. Then there should be an interim period of review of cases going forward under the recently modified SOP.

The Panel is aware that DNA analysts in the FSL of DFS had received some training on DNA mixture interpretation and statistical calculations by selected members of the DFS Advisory Board in the summer of 2014 and again in early 2015. A review of these training materials confirmed that basic DNA mixture interpretation was included but there were few if any examples of the case specific concerns identified by the Panel in its review of selected casework.

Use of a new worksheet to carefully document steps in the DNA interpretation pipeline is a welcomed and very good step forward and should reduce chances for cognitive (interpretation) bias. The Panel notes that additional recent internal training at DFS was designed to familiarize the analysts with the new mixture interpretation protocols and documentation worksheets. However, going forward it will be important that additional training be focused on the issues raised by the USAO and the Panel so that the areas of specific concern can be fully addressed.

The Panel reviewed the DNA profile used by DFS for final evaluation of the DNA analysts. The Panel finds that the "test" utilized does not constitute an adequate assessment of each analyst's ability to properly evaluate and interpret DNA mixture evidence. Serious consideration should be taken to develop and deliver tests that effectively assess each analyst to help assure that the above protocols, procedures, review processes, and continuing education enable DFS staff to attain high quality DNA typing results.

2) Internal Quality Improvement Program Needed. Open discussion of the concerns of the USAO and the Panel is needed with the DNA analysts, technical leader, supervisors, and management. DNA analysts may have had cogent reasons for how they proceeded in individual cases or their practices may not be acceptable. It is most productive to have communication with all practitioners when assessing the root cause of potential problems.

3) Improvements in Technical Review: The Panel was informed that DFS plans to perform group technical reviews of upcoming DNA mixture cases, for an unspecified time period, using its new interpretation protocol. Review of this process will be needed to assess whether such changes will address the concerns and issues raised to date by the Panel and the USAO. In addition, review of the process should address any documentation requirements for such group technical reviews.

4) Validation of Analytical Thresholds. As noted above the process described appears, to the Panel, to be inappropriate and needs more attention before final implementation.

5) Revisit policy on minimum # of loci for DNA mixture statistics. This matter warrants discussion with customers and should be addressed on a case-by-case basis.

6) Audit of past cases. Review of additional previous casework regarding DNA mixture analysis is warranted to identify items in which departures from recommended interpretive practices exist and which were not identified during the non-exhaustive review by DFS or by the Panel. Specific attention will be needed in the review of cases in which the 29 cycle protocol was used.

7) Training and continuing education for upper management. Continuing education and management training should be encouraged for upper management and supervisors to include topics on DNA analysis, quality assurance practices and root cause analysis. Upper management should communicate more effectively with its customers and DNA analysts regarding any concerns raised by its clients and advisors. Such communications and deliberations should be carefully documented and all material changes in its SOP (e.g., 29 cycle PCR) should be communicated in a timely fashion to its key clients.

Suggested Readings

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APPENDIX

Case Examples

United States v David Shepherd, 2012CF1009602, DFS Lab# M140247

In US v Shepherd DFS concluded that Henry Miller (item 5.0) could not be excluded as a potential contributor to DNA extracted from a weapon swab (items 3.1W_3.1D). DFS applied a CPI calculation and obtained estimates of the portion of the population that could be included that ranged from 1 in 368,000 to approximately 1 in 1 million. Intuitively, for Henry Miller to be included as a potential contributor then the alleles he carries also should be observed at the loci included in the CPI. Otherwise the calculation has no relevance regarding an association of Henry Miller and the evidence item. Based on the tables in the laboratory report and the electropherogram, DFS applied the CPI to the following loci D7S820, D3S1358, D2S1338, D19S433, vWA, and TPOX. At D7S820 the evidence item displayed 8,12 and Henry Miller displayed 10,12; at D2S1338 the evidence item displayed 17,20 and Henry Miller displayed 17,23; and at TPOX the evidence item displayed 9,11 and Henry Miller displayed 6,8. For Henry Miller to be included and a CPI rendered DFS must assume that allele drop out has not occurred at these three loci. However, the CPI calculation at these three loci did not include the 10 allele at D7S820, the 23 allele at D2S1338, and the 6 and 8 alleles at TPOX that are present in DNA from Henry Miller's known sample. If the laboratory used the CPI at these loci under the assumption that no allele drop out has occurred then Henry Miller would have been excluded as a potential contributor. Yet, DFS and the Panel concur that Henry Miller cannot be excluded as a potential contributor to the DNA mixture. The laboratory report attributes the major contributor of the mixture of items 3.1W_3.1D to an unknown male. Therefore, DFS can only reasonably include Henry Miller as a potential minor contributor. The laboratory results indicate that alleles at the three loci (D7, D2, TPOX) are derived from the "major" contributor and that the "minor" contributor alleles potentially have "dropped out". Therefore, DFS rendered a CPI mixture calculation on portions of the evidence where only a single source is the most plausible explanation of the profile and by the laboratory's own statement the alleles are not attributed by Henry Miller. In essence, DFS has provided a statistical calculation of the portion of the population that could potentially contribute to the mixture of item 3.1W_3.1D in which Henry Miller is not part of the portion of that population.

It is a reasonable expectation that alleles from the "minor" contributor of items 3.1W_3.1D could be dropping out at a number of loci. Yet, DFS failed to take into consideration the potential of allele dropout of the minor contributor at the loci D3S1358 and vWA. The outcome is that of the six loci that were used to calculate the CPI only one marker should have been considered. The strength of the evidence will be substantially reduced.

The Panel concludes that DFS has not interpreted the evidence correctly for this item in this case to account for allele drop out and should more carefully evaluate allele profiles of evidence and reference samples when an inclusion is made.

United States v. George Cocroft, 2012-CF1-20633, DFS Lab # M130061-1

In this particular case DFS concluded that the Brown (victim) and Cocroft (defendant) cannot be excluded as possible contributors to the mixture from item 1A.1SF, a vaginal swab. However, DFS calculated a CPI only for Cocroft and despite Brown's inclusion in the mixture no CPI was calculated regarding Brown as a possible contributor. The computation of a CPI statistic for one possible contributor to a mixture and not another shows a fundamental lack of understanding regarding CPI calculations. Presumably no statistical calculation was associated with Brown is because some of Brown's alleles are not observed at loci used for the CPI related to Cocroft. Thus, allele dropout must have occurred to explain Brown as a contributor. The CPI statistical results in reference to the mixture obtained from Item 1A.1SF failed to address the potential for drop out. Specifically, DFS calculated a CPI at the D3S1358 and D13S17 loci. At D3S1358, DFS called "17,17" alleles, but Brown is a "15, 17" at that locus. Thus, Brown's 15 allele would have had to drop out to be included as a contributor. At D13S17, DFS called "11,11" alleles, but Brown is a "12, 13" at this locus; again indicating allele drop out to explain Brown as a contributor. To conduct a CPI calculation at these two loci, DFS would have had to exclude Brown from her own intimate sample. The panel notes that the DNA profile of Brown was present in the epithelial fraction of the same vaginal swab.

A prominent inconsistent practice identified by the Panel in review of selected cases was not subtracting out the victim contribution from a vaginal swab and yet in other cases intimate samples subtraction was performed. The use of CPI in this case stems from DFS failing to subtract out the alleles of Brown from her own intimate sample (despite that fact that DFS's protocols permit subtraction of a known contributor from a mixture). In fact, the sample given in the DFS protocol is subtracting out a victim's profile from the DNA profile of the sperm fraction from a vaginal swab. While not subtracting the victim profile from an intimate sample is within acceptable variation and one might agree with this position, there were a number of examples in the cases reviewed by the Panel where subtraction was performed and this case was the only one identified where it was not performed. The Panel notes if subtraction was performed the statistical calculation would have resulted in a value of 1 in 900 million using the RMP. While the Panel would have chosen to perform a subtraction, it does not take a position on whether this is within acceptable interpretation variation. Instead the Panel points out that the practices of subtraction varied within the same lab, which is an indication of a lack of consistent technical review in the DNA analysis pipeline.

United States v. Breal Hicks, et al., 2013-CR-203 (RJL), DFS Lab # M130107-1

DFS concluded that a male profile was obtained from the dry swab from the magazine within a .380 cal pistol (Item 40) and that "Dwayne Brown (Item 58.1) cannot be excluded as a possible contributor of this profile. All other submitted known samples were excluded as possible contributors of this profile. DFS then calculated the RMP for an unrelated, random individual having a STR profile which cannot be excluded as a contributor of the DNA profile obtained from the dry swab from Item 40. It is noted that DFS did not declare explicitly the profile developed from Item 40 was a mixture, although the profile clearly is a mixture. In addition, the

29-cycle amplification protocol (discussed above) was used for analysis of Item 40. Based on the results Brown should have been excluded from the profile from Item 40. The Panel was concerned that interpretation bias might be part of the explanation for the inclusion - based on the known profile of Brown - even though there are inconsistent results between the evidence and the known profile of Brown at the D8S1179 and TH01 loci. Because DFS employed a 29 cycle amplification the ST is set at 300 rfu. The ST of 300 rfu is supposedly based on validation studies by DFS. Therefore, a single peak at a locus would be deemed a homozygote as there should be a very low probability that allele drop out would have occurred (which is the purpose of establishing ST). DFS reported a genotype assignment at the D8S1179 locus as a "15,15" for item 40, indicating that DFS interpreted the genotype as a homozygote, as it should have based on its established ST. In contrast, the known reference sample of Brown has two alleles - a 12 and a 15 at the D81179 locus. That is, Brown is a heterozygote, not a homozygote at the D81179 locus. Also, DFS labeled the TH01 locus for item 40 as "6,(8)" and the known profile of Brown has only a 6 allele (no 8 allele) at the TH01 locus. As the DNA profile for Item 40 was not described by DFS in the report as a mixture, the TH01 profile from item 40 cannot be interpreted as consistent with that of Dwayne Brown. One might argue that the peak height ratio (PHR) for the 6 and 8 allele at the TH01 locus in item 40 is below 50% (i.e., 48.1%) and that could explain the reason for not including the locus in the RMP calculation. However, DFS routinely has used lower PHRs for the RMP. More importantly, if the 8 allele was rejected as part of the major profile then the sample should have been declared a mixture. Based on DFS's interpretation guidelines, and the other considerations described above, it was the opinion of the Panel that Dwayne Brown should have been excluded as a potential contributor of this particular item.

The Panel opined that bias could have factored into the interpretation by DFS because the RMP did not include inconsistent loci (and particularly not including the D8S1179 data in the statistical calculation performed by DFS). Simply removing inconsistent loci from a statistical calculation, in and of itself, is not necessarily a conservative practice (especially if the data are exculpatory). The data are discordant based on DFS interpretation guidelines, and DFS should have reported the discrepancies in its report (and at a minimum in its case file). The practices described in this case are not, in the opinion of the Panel, in accordance with accepted practices within the scientific community when conducting statistical analyses of forensic DNA mixtures.

United States v. Mohammed Roble, 2013-CF1-6095, DFS Lab # M130206-1

DFS concluded that Tiblets and Roble cannot be excluded as potential contributors to the DNA mixture obtained from Roble's hand swab item 1_2 (a swab from back of left hand and fingers/palm side left hand and fingers). For reasons unclear to the panel, the DFS calculated two separate CPIs for this exemplar - one calculation using allele calls at locus D16S539 and one NOT using allele calls from locus D16S539. The allele calls for the mixture profile were 10,11. The known profile of Roble shows a 9,11 at the D16S539 locus. Since DFS included Roble in the mixture, the fact that there is no indication of a 9 allele DFS must assume that complete allele drop out occurred. Applying a CPI that fits only Tiblet's profile does not take into account allele drop out and misstates the portion of the population that could contribute to the mixture (at the loci used for the CPI). Calculating two CPIs is inconsistent with the concept of applying the CPI.