THE DANGERS OF NOT ASSUMING CONTRIBUTORS – WHY THE GOAL OF "CONSERVATIVE" IN FORENSIC DNA STATISTICS SHOULD BE DROPPED IN FAVOR OF BEING "INFORMATIVE"

Joel Sutton, MSFS, Tim Kalafut, PhD, Lindsey Smith, MSFS, Curt Schuerman, BSc, David Diekema, MSFS, United States Army Criminal Investigation Laboratory,
4930 North 31st Street, Forest Park, GA 30297

Correspondence should be sent to Joel Sutton, joel.d.sutton2.civ@mail.mil

Introduction

Evaluating the statistical weight for a person of interest (POI) being included in a DNA mixture can be difficult. The conventional statistical method mostly used today (i.e., the combined probability of inclusion or exclusion) is simple to calculate, easy to explain in court, and is said to be "conservative" to the accused since it makes no assumptions with the number of contributors or with conditioning on a known contributor genotype [1, 2]. This leads to including more genotypes in the calculation meaning more random unrelated individuals would be included, which equates mathematically to very low numbers being reported. Alternative methods (i.e., likelihood ratio, random match probability) are available which better explain what genotypes likely make up the mixture, but these can involve complex formulae, are said to be more difficult to explain in court, and do require assumptions as to the number of contributors [2,3,4]. They also allow for restricting possible genotype combinations based on assuming known contributors.

More recently probabilistic genotyping has been advocated as a better model for interpreting complex mixtures (i.e., more than two contributors, with stochastic contributors present) [5]. Some of these models offer quantitative genotyping which utilizes peak height information to further model and weight likely genotypes for a person of interest in a mixture [6-8]. As a result, these models will yield a very informative statistic (i.e., high number) that may be construed as being less conservative than with conventional methods.

Although making assumptions to the number of contributors in a mixture or assuming genotypes from known donors (e.g., owner of the item) in a mixture is a common practice, it is not always consistently applied. Often, the decision to assume is dependent on individual laboratory policy or "comfort level" with the individual DNA analyst. When is it "reasonable" to assume versus not? Making assumptions may also be perceived as anti-conservative or biased against the accused in some way. However, we propose that there are some instances when not making assumptions for known contributors in a mixture will not benefit the accused at all. This proposition was tested using empirical data and the results will be discussed in this manuscript. Our results reject the notion that a conservative statistic is useful or somehow necessary for the court; but rather that the goal should be to provide a statistic that is most informative to the court in assisting them with evaluating the presence of a person of interest in the mixture. Additionally, laboratories wishing to move to probabilistic genotyping from the combined probability of inclusion/exclusion must understand that it uses a likelihood ratio model so assumptions will be needed and even preferred in many instances.

Methods

Consider the following mock scenario:

An alleged victim was abducted from bar while intoxicated and sexually assaulted in her car in the parking lot. The investigation revealed a suspect that was seen in the bar that night. The back seat of the car owned by victim was examined for evidence and a semen stain was found. The stain yielded a DNA mixture shown in Figure 1.

This DNA profile was generated in the laboratory using Identifiler Plus™ on a 3130xl. It was created to be a two contributor mixture with contributor proportions at 1:1 or 50/50. All of the alleles from the suspect and victim are present in the profile, with nothing extra. Two questions were then posed. What is the best approach for interpretation and statistics? What is the impact of assuming a known contributor (i.e., the victim on her own car seat) in evaluating the presence of the suspect in this mixture?

The following statistical methods were utilized to answer these questions: combined probability of inclusion (CPI), random Match Probability (RMP), and unconstrained combinatorial likelihood ratio (ucLR), and a fully continuous probabilistic genotyping likelihood ratio (LR). ArmedXpert™ software was utilized for calculating the CPI, RMP, and ucLR. STRmix™ was utilized for calculating the probabilistic genotyping LR. Two interpretations were evaluated for each method: assuming the victim (it's her car) and not assuming the victim. Comparisons were made at the locus level (Test 1) and then for the entire mixture (Test 2).

For simplicity, the mixture was also assumed to be well above the laboratory-defined rfu stochastic threshold, so that all methods could be fully optimized. Additionally, theta was set to zero to try and level out some of the differences in the different approaches (i.e. NRCII 4.1 v 4.2, CPI does not use theta, etc.) [9]. For the likelihood ratio methods, the proposition for the numerator (H1) was that the victim and suspect were the contributors; otherwise, where's the crime? Only the point source LR was used for the STRmix™ LR. The NIST 1036 Caucasian allele frequencies were used for all calculations [10].

Results

Test 1 – The TH01 locus (Figure 2) was used for this assessment. The victim's genotype is 7,9 and the suspect's genotype is 6, 9.3. The CPI, RMP, and ucLR were calculated for this locus with the victim not assumed, then re-calculated with the RMP and ucLR assuming the victim's genotype (i.e., the victim's genotype in the LR was assumed in H1 and H2). The mixture was also assumed to be from two contributors using the RMP and ucLR. Results are shown in Table 1.

The following observations from Test 1 were made in terms of the statistics for TH01. The CPI approach allowed for all possible genotype combinations. The RMP approach with no assumptions allowed for all heterozygous genotypes but removed the homozygous combinations based on the assumption of two contributors. The RMP assuming the victim on the car seat of her vehicle further restricted combinations to a single genotype possibility. Thus, the RMP assuming the victim was the most informative among the three approaches as to the true genotype, therefore "good" for the innocent, and "bad" for the guilty. The CPI gave the most conservative result but because it allowed for more genotype possibilities, it is considered "good" for the guilty (in terms of the number) but "bad" for the innocent. In other words, a "conservative" statistic is only beneficial to the accused if you are truly guilty. However, it includes lots of other genotype possibilities. In reality, only one type fits the data and the scenario, meaning if you're innocent and are not a 6,9.3 genotype at that locus but have some other combination of 6,7,9,9.3 it would

not be beneficial for you with a CPI approach as you could be falsely included. Being "conservative" is great, right up until you include an innocent person.

In terms of the ucLR comparisons, the more "conservative" number was to assume the victim rather than not. This was due to how the proposition was formulated. We would assert that the victim and suspect best explain the mixture; therefore they must be in the prosecution's hypothesis (H1). Otherwise, what is the relevance of the evidence? Additionally, minimizing the number of contributors in the H2 to align with H1 based on the observed data was also conducted with this experiment. Otherwise, depending on who has the rare alleles, proposing more unknown contributors in the H2 than in H1 could certainly be detrimental to the accused in terms of the resulting LR [11]. The ucLR is more conservative when you assume the victim on her own car seat, and it is more informative, as it limits the possible genotypes for the unknown contributor in H2.

Test 2 – The entire profile (Figure 1) was used for this assessment. The CPI, RMP, ucLR, and STRmix™ LR were calculated with the victim not assumed, then re-calculated with the ucLR, RMP, and STRmix™ LR assuming the victim's genotype (i.e., the victim's genotype in the LR was assumed in H1 and H2). The mixture was also assumed to be from two contributors using the RMP, ucLR, and STRmix™ LR. Results are shown in Table 2.

The following observations from Test 2 were made in terms of the overall statistics for the mixture. The most conservative but least informative statistic was the CPI approach. The RMP assuming the victim was less conservative in terms of the number but more informative than the CPI and RMP with no assumptions. It was also less conservative but more informative than the ucLR assuming the victim because the RMP approach utilized here applies peak height information to further restrict genotypes (i.e., quantitative genotyping).

In terms of the LR approaches, the ucLR with no assumptions was less conservative than the ucLR with the victim assumed but less informative since it allowed for more genotype combinations for the unknown contributor in H2. The STRmix™ LR was the least conservative since it uses quantitative (peak height) information to give weight to more likely genotype possibilities, eliminating some combinations thus becoming more informative. When assuming the victim, the STRmix™ LR was more conservative than the ucLR and STRmix™ LR where no assumption for the victim was made.

Test 3 – The entire profile (Figure 1) was used for this assessment. The RMP approach was compared to the STRmix™ LR where the victim was assumed for both approaches. This time the RMP was further refined by applying proportions based on peak height ratio expectations. Results are shown in Table 3.

The following observations from Test 3 were made in terms of the overall statistics for the mixture. In evaluating the data, it was noted that STRmix™ added in four extra genotype possibilities for the contributor foreign to the victim. Even though they had < 0.1% weight, three of those four genotypes involved more rare alleles than the fully deduced contributor from the RMP statistic. Thus with a STRmix™ LR, the relative "rarity" of alleles can have an impact on the LR in unexpected ways (making it less conservative for a different reason). Overall, the refined RMP approach was mostly comparable to the STRmix™ LR with the statistic.

Conclusions

With an LR, it is never conservative when there are no assumed contributors. When it is reasonable to assume known contributors, there will be no difference with an ucLR and an RMP. Based on these results, the goal should not be to calculate a "conservative" number; rather the goal should be to calculate

an “informative” number. An informed statistic and interpretation may be less conservative in terms of the number but drastically minimizes the risk of a false inclusion since it utilizes the most information based on the data. With an informative result, the “number” calculated is consistent with the genotypes that make sense, and therefore is consistent with the overall interpretation of results.

These results demonstrate that making reasonable assumptions not only assists with the mixture deconvolution, but also leads to a more informative result. Any assumptions should be clearly stated in the report for full disclosure. Since a CPI approach does not make assumptions for known contributors, it is limited in providing informative results. The ucLR, RMP, and probabilistic LR (using STRmix™) will also allow for more genotype combinations when no assumptions for a known contributor are made. It is our contention based on these results that the most informative results for both the prosecution and defense is the RMP or STRmix™ LR that both assumes known contributors where appropriate and utilizes quantitative genotyping for maximizing the exclusionary power of DNA results. We would expect all parties at trial to desire the more informative statistic which best aligns with the associated interpretation.

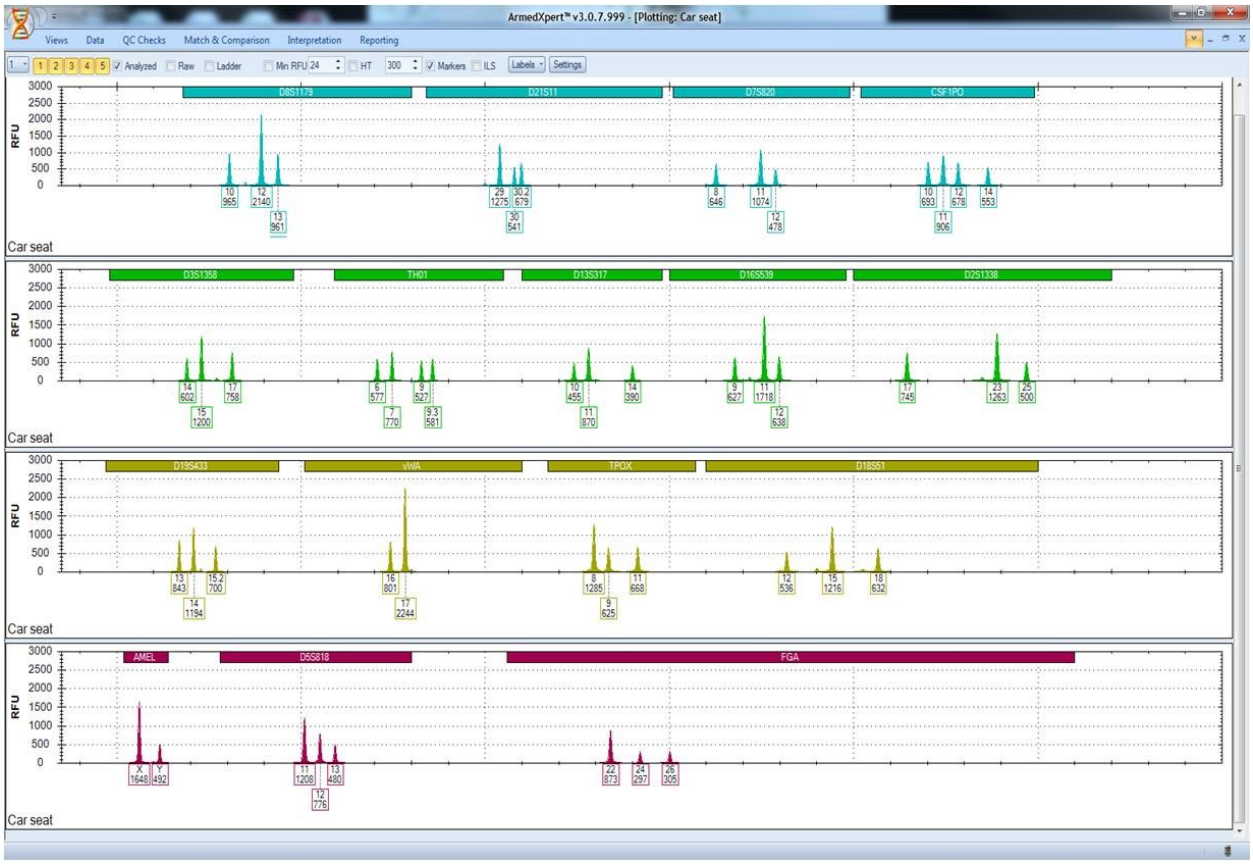

Figure 1

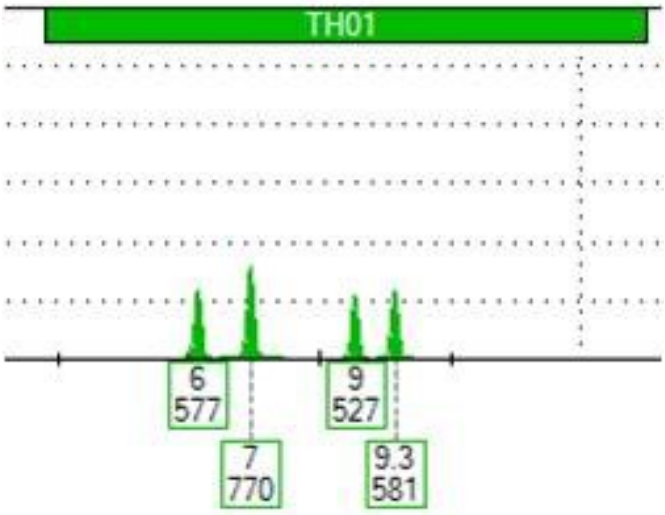

Figure 2

Table 1 – TH01 evaluation	
Not assuming the Victim	**Assuming the Victim**
CPI = 1 in 1.2	**Not applicable, same calculation**
RMP = 1 in 1.7	**RMP = 1 in 6.1**
ucLR = 22 times more likely	**ucLR = 6.1 times more likely**

Table 2 – Entire mixture evaluation	
Not assuming the Victim	**Assuming the Victim**
CPI = 1 in 11 million (1E7)	**Not applicable, same calculation**
RMP = 1 in 100 million (1E8)	**RMP = 1 in 39 quadrillion (1E16)**
ucLR = 2.6 septillion (1E24) times more likely	**ucLR = 6.1 times more likely**
STRmix™ LR = 140 octillion (1E28) times more likely	**STRmix™ LR = 2.6 septillion (1E24) times more likely**

Table 3– Entire mixture evaluation (quantitative genotyping) - assuming victim	
RMP – restricted approach using phr and proportions	**STRmix™ LR**
1 in 160 quadrillion (1E16)	**170 times more likely (1E17)**

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