Title: Comparing multiple POI to DNA mixtures.

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Abstract

In casework, laboratories may be asked to compare DNA mixtures to multiple persons of interest (POI). Guidelines on forensic DNA mixture interpretation recommend that analysts consider several pairs of propositions; however, it is unclear if several likelihood ratios (*LR*s) per person should be reported or not. The propositions communicated to the court should not depend on the value of the *LR*. As such, we suggest that the propositions should be functionally exhaustive. This implies that all propositions with a non-zero prior probability need to be considered, at least initially. Those that have a significant posterior probability need to be used in the final evaluation. Using standard probability theory we combine various propositions so that collectively they are exhaustive. This involves a prior probability that the sub-proposition is true, given that the primary proposition is true. Imagine a case in which there are two possible donors: *i* and *j*. We focus our analysis first on donor *i* so that the primary proposition is that *i* is one of the sources of the DNA. In this example, given that *i* is a donor, we would further consider that *j* is either a donor or not. In practice, the prior weights for these sub-propositions may be difficult to assign. However, the *LR* is often linearly related to these priors and its behaviour is predictable. We also believe that these priors are unavoidable and are hidden in alternative methods.

We term the likelihood ratio formed from these context-exhaustive propositions $LR_{i/\bar{i}}$.

$LR_{i/\bar{i}}$ is trialled in a set of two- and three-person mixtures. For two-person mixtures, $LR_{i/\bar{i}}$ is often well approximated by $LR_{ij/ja}$, where the subscript *ij* describes the proposition that *i* and *j* are the donors and *ja* describes the proposition that *j* and an alternate, unknown individual (*a*), who is unrelated to both *i* and *j*, are the donors. For three-person mixtures, $LR_{i/\bar{i}}$ is often well approximated by $LR_{ijk/jka}$ where the subscript *ijk* describes the proposition that *i*, *j*, and *k* are the donors and *jka* describes the proposition that *j*, *k*, and an unknown, unrelated (to *i*, *j*, and *k*) individual (*a*) are the donors. In our simulations, $LR_{ij/ja}$ had fewer inclusionary *LR*s for non-contributors than the unconditioned *LR* ($LR_{ia/aa}$).

1.0 Introduction

With improvements to the sensitivity of forensic DNA analysis methods, mixed DNA profiles are often recovered from forensic exhibits and crime scenes. Depending on the case circumstances, a person or persons of interest (POI) may need to be compared with these mixed profiles. As shown in multiple guidelines (detailed in section 1.2), likelihood ratios (*LR*s) are the preferred method of quantifying the value of these comparisons. These *LR*s may or may not support the inclusion of the POI as donors to the DNA mixture. With multiple POI and vague case information, it is often difficult to formulate appropriate propositions regarding the source of the DNA: does the DNA originate from all POI? From one only? From none? All possibilities that are meaningful to the decision maker should be considered. DNA commissions have recommended that an *LR* is reported for each POI. This

is particularly important when the DNA mixture is unbalanced. For investigative purposes, the International Society of Forensic Genetics DNA Commission recommends checking if all given POI in combination explain the recovered DNA profile [1]. But what if they do not? Shall one report several *LR*s for the same person? If so, how is the decision maker to make sense of these *LR*s? For this reason, we suggest the use of multiple propositions that are collectively exhaustive within the context of the case.

1.1 Two-person mixture

Consider a case where DNA has been analysed and compared to two individuals, P_1 and P_2. As recommended in the literature, we assign the value of the comparisons for P_1 and P_2 separately versus together. At least four different propositions seem reasonable unless case circumstances suggest otherwise:

H_{12}: the DNA originated from P_1 and P_2

H_{1a}: the DNA originated from P_1 and an unknown person unrelated to P_1 or P_2

H_{2a}: the DNA originated from P_2 and an unknown person unrelated to P_1 or P_2

H_{aa}: the DNA originated from two unknown persons unrelated to P_1, P_2, or each other.

A likelihood ratio considers the probability of the evidence with respect to pairs of propositions (sometimes referred to as hypotheses [*H*]). The following proposition pairs have been discussed previously [2]: (H_{1a}, H_{aa}), (H_{2a}, H_{aa}), (H_{12}, H_{1a}), (H_{12}, H_{2a}), and (H_{12}, H_{aa}). The *LR*s corresponding to these proposition pairs are noted:

$LR_{1a/aa}$, $LR_{2a/aa}$, $LR_{12/1a}$, $LR_{12/2a}$, and $LR_{12/aa}$, where the subscript describes which pair of propositions are being considered.

1.2 Existing guidance

We review existing published guidance on assigning *LR*s when there are multiple POI and on conditioning on the genotypes of one or more POI as assumed donors to the mixture.

1.2.1 Forensic Science Regulator

The Forensic Science Regulator DNA mixture guidelines [3] (hereafter FSR guidelines) state:

Clause 6.8.17: "Assume that the questioned profile may be reasonably taken to be a mixture of two genotypes. There are two POIs and the questioned profile consists of peaks that correspond to the alleles in the suspects' genotypes and no others. Then it is tempting to address propositions of the following kind.

> H_p: The DNA is a mixture of persons of interest 1 and 2 (POI 1 and POI 2).
>
> H_d: The DNA is a mixture of two unknown people, unrelated to POI 1 and POI 2."

However, as noted, it would seem wrong to assign the same value to each comparison, in particular with unbalanced DNA mixtures.

Clause 6.8.18: "At the very least, the scientist could be expected to consider a calculation for each of the following two prosecution propositions.

> H_p: The DNA is a mixture of person of interest 1 (POI 1) and an unknown person who is unrelated to POI 1.
>
> H_p: The DNA is a mixture of person of interest 2 (POI 2) and an unknown person who is unrelated to POI 2."

Clause 6.8.19: "Each (of the above H_p propositions) would be considered with the same defence proposition as before.

> H_d: The DNA is a mixture of two unknown people, unrelated to POI 1 and POI 2."

This suggests that $LR_{1a/aa}$ and $LR_{2a/aa}$ should be assigned.

We will term this set of likelihood ratios

FSR set 1: $LR_{12/aa}$

FSR set 2: $LR_{1a/aa}$ and $LR_{2a/aa}$

Regarding $LR_{12/aa}$, clause 6.8.20 states: "if both *LR*s support the prosecution propositions it is still conceivable that the first pair of propositions [FSR set 1] lead to a *LR* of less than one, so the calculation for that pair should be checked and reported."

Clause 6.8.21: "In the event that one of the POIs later pleads guilty, the scientist may be invited to repeat the interpretation conditioning on the presence of that POI's genotype.

> H_p: The DNA is a mixture of persons of interest 1 and 2 (POI 1 and POI 2).
>
> H_d: The DNA is a mixture of person of interest 2 (POI 2) and an unknown person unrelated to POI 1 and POI 2."

This could suggest that $LR_{12/2a}$ should be assigned only in this circumstance.

We will term this set of likelihood ratios

FSR set 3: $LR_{12/1a}$ and $LR_{12/2a}$

1.2.2 American Academy of Forensic Sciences Standards Board

The draft American Academy of Forensic Sciences Standards Board standard regarding assigning propositions for *LR*s [4] (hereafter ASB) agrees partially with the FSR guidelines:

> Clause 4.5: "Where multiple POIs have *LR*s that support an association to a DNA mixture, within the capabilities of the approach used, an analysis shall be performed using proposition pairs that test whether the multiple POIs can be included together in the observed DNA profile."

> Clause A.5: "The analysis should separate the propositions into their simplified constituents (i.e., simple proposition pairs[1]) when an *LR* favoring H_1 has resulted from a compound proposition pair[2] incorporating multiple POIs under H_1 and none of the POIs under H_2, in order to establish the weighting and the consequent probative value of the evidence per contributor under H_1."

These two clauses suggest the calculation of one of the following sets of *LR*s:

ASB Set 1: $LR_{1a/aa}$, $LR_{2a/aa}$, and $LR_{12/aa}$ or

ASB Set 2: $LR_{12/1a}$, $LR_{12/2a}$, and $LR_{12/aa}$

The guideline on assuming conditioning profiles embraces broader use of this valuable tool:

> Clause 4.4: "The laboratory shall have a documented policy defining when a conditioning profile will be used. Support for the assumption of non-intimate conditioning contributors shall be documented in the case file."

1.2.3 Gittelson et al. [2]

Gittelson et al. explicitly discuss the options in ASB sets 1 and 2 above but make the point that, if P_1 and P_2 fully explain the profile, then the prosecution proposition will logically be:

H_{12}: P_1 and P_2 are the donors to the DNA,

which leads directly to the use of FSR set 3.

1.2.4 The DNA Commission of the International Society of Forensic Genetics (hereafter ISFG) have also offered recommendations for the evaluation of forensic DNA typing results at the (sub-) source level [1]. Their recommendation 3 states:

> "When the issue regards the possible presence of DNA from several persons of interest, effort should be made to evaluate the profiles separately, and not as a whole. This is especially important if the information available from one part of the profile (e.g. major) is different from the other (minor, partial). For evaluation, this can be achieved by considering the result of the comparison between the given person and the trace and calculating individual *LR*s for each person. The report should be fully transparent on what propositions have been considered and on what basis."

Within [1], the following sets of *LR*s are endorsed:

ISFG Set 1: $LR_{1a/aa}$ and $LR_{2a/aa}$ or

ISFG Set 2: $LR_{12/1a}$ and $LR_{12/2a}$

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[1] Simple proposition pair: A pair of propositions where no more than one POI in H_1 is replaced with an unknown donor in H_2 or vice versa

[2] Compound proposition pair: A pair of propositions where more than one POI in H_1 is replaced with unknown donors in H_2 or vice versa

Recommendation 3 continues and states:

> "For investigative purpose, it might be useful to explore whether the results support the proposition that the two persons together are (or not) the source of the DNA. In such a case, one can assign one *LR*."

This would seemingly advocate the use of $LR_{12/aa}$ for investigative purposes only and not for evaluative purposes (i.e. reporting in court). However, this recommendation should not be read as an endorsement to omit that the two POI can (or cannot) both be donors, as the court may value knowing this. If the persons both explain the mixture and $LR_{1a/aa}$ and $LR_{2a/aa}$ are larger than one, the results are not difficult to understand. However, we would expect considerable difficulty for the court to make sense of the findings in circumstances where the persons do not jointly explain the mixture (i.e. $LR_{12/aa}$ equals zero) yet $LR_{1a/aa}$ and $LR_{2a/aa}$ are each larger than one. We argue that assigning a single *LR* that considers all meaningful sub-propositions would add value to the process.

1.3 Criticism of non-exhaustive propositions

There has been some criticism that the propositions commonly used in forensic DNA interpretation are not exhaustive. For example, Stiffelman [5] says "Not only are there multiple alternative hypotheses that could explain the evidence, but both hypotheses in the equation could in fact be wrong, and there would still be an *LR* reported."

Fenton et al. [6] state "When the assumption of mutually exclusive and exhaustive hypotheses is either wittingly or unwittingly undermined, the relationship between the *LR* and the notion of 'probative value' of the evidence can change dramatically."

1.4 Propositions that are exhaustive, based on the context of the case

LR sets 1 and 2 of ASB or ISFG are assigned given mutually exclusive but not collectively exhaustive propositions. They are open to a number of criticisms.

In order to conform to the principles of interpretation, one needs to consider the case information that has an impact on the value of the forensic result and the formulation of propositions. The case information encompasses the fact that the laboratory was asked to compare the DNA mixture with several POI. One might assume that this means that each POI has a non-zero prior probability of being the donor. Unfortunately, samples are sometimes submitted from one or more POI who were known to be unlikely donors, for instance people who were overseas at the time of the offence. In one example, samples from 27 members of a pedigree were submitted for examination in the homicide of five people from the same pedigree in Sydney, Australia. Many of the pedigree members whose samples were submitted were not in Australia at or around the time of the homicide.

For defendant P_1, the alternate proposition will generally assume that he is not a donor. As there is another POI, we suggest considering at least the union of H_{2a} and H_{aa}: that is, the DNA is not from P_1 but may or may not be from P_2.

144 It is helpful to define the proposition space $H = \{H_{12}, H_{1a}, H_{2a}, H_{aa}\}$. We can then define the
145 compound propositions $H_1 = \{H_{12}, H_{1a}\}$ for P_1 and $H_2 = \{H_{12}, H_{2a}\}$ for P_2 and their
146 respective complements $H_{\bar{1}} = \{H_{2a}, H_{aa}\}$ and $H_{\bar{2}} = \{H_{1a}, H_{aa}\}$. These compound
147 propositions are mutually exclusive and exhaustive within the context of the case (assuming
148 that there are exactly two contributors and discounting the possibility of related donors).
149 Therefore, the joint probability of any of these compound propositions can be expressed as
150 the sum of the individual propositions. That is, $\Pr(H_1) = \Pr(H_{12}) + \Pr(H_{1a})$

151 $\Pr(H_{\bar{1}}) = \Pr(H_{2a}) + \Pr(H_{aa})$

152 $\Pr(H_2) = \Pr(H_{12}) + \Pr(H_{2a})$

153 and $\Pr(H_{\bar{2}}) = \Pr(H_{1a}) + \Pr(H_{aa})$

154 In the following $LR_{i/j}$ considers the propositions *i* and *j*.

155 *I* represents the case-relevant information (sometimes termed the 'framework of
156 circumstances').

157 We can consider the value of the DNA results given that P_1 is a donor regardless of whether
158 or not P_2 is also a donor by calculating:

159 $$LR_{1/\bar{1}} = \frac{\Pr(E \mid H_{12}, I)\Pr(H_{12} \mid H_1, I) + \Pr(E \mid H_{1a}, I)\Pr(H_{1a} \mid H_1, I)}{\Pr(E \mid H_{2a}, I)\Pr(H_{2a} \mid H_{\bar{1}}, I) + \Pr(E \mid H_{aa}, I)\Pr(H_{aa} \mid H_{\bar{1}}, I)}$$ (equation 1)

160 since $\Pr(E \mid H_{12}, H_1) = \Pr(E \mid H_{12})$, $\Pr(E \mid H_{1a}, H_1) = \Pr(E \mid H_{1a})$,
161 $\Pr(E \mid H_{2a}, H_{\bar{1}}) = \Pr(E \mid H_{2a})$, and $\Pr(E \mid H_{aa}, H_{\bar{1}}) = \Pr(E \mid H_{aa})$.

162 The terms of the type $\Pr(H_{12} \mid H_1, I)$ are the probability that H_{12} is true given that H_1 is true
163 and considering the case-relevant information, *I*. We feel that, given sub-source propositions,
164 there is likely to be very little information in *I* that helps inform these probabilities in most
165 cases. In such cases it seems reasonable to assume

166 $\Pr(H_{12} \mid H_1, I) = \Pr(H_{1a} \mid H_1, I) = \Pr(H_{2a} \mid H_{\bar{1}}, I) = \Pr(H_{aa} \mid H_{\bar{1}}, I) = \frac{1}{2}$ (assumption 1).

167 This leads to:

168 $$LR_{1/\bar{1}} = \frac{\Pr(E \mid H_{12}, I) + \Pr(E \mid H_{1a}, I)}{\Pr(E \mid H_{2a}, I) + \Pr(E \mid H_{aa}, I)}$$ (equation 2).

169 Although assumption 1 leads to equation 2, it is not necessary. As long as the four
170 probabilities are equal, equation 2 follows. However, in order for the propositions to be
171 exhaustive and obtain equation 2, assumption 1 is needed. In general this would seem
172 reasonable but it would be best if this was disclosed in some way.

If the probabilities in equation 2 are available, it is desirable to use them directly. However, many software do not provide these but instead provide an *LR* without ever having available the numerator or denominator probabilities. For these software, $LR_{1/\bar{1}}$ can be assigned in a number of ways.

We begin by considering $LR_{1/\bar{1}} = \frac{LR_{12/aa} + LR_{1a/aa}}{1 + LR_{2a/aa}}$ (equation 3).

If the evidence supports the inclusion of the two POI alone and together then $LR_{12/aa} > LR_{1a/aa} > 1, LR_{2a/aa} > 1$ and equation 3 becomes approximately

$LR_{1/\bar{1}} = \frac{LR_{12/aa}}{LR_{2a/aa}} = LR_{12/2a}$.

Consider the case where the *LR*s support the inclusion of one suspect and the exclusion of the other. Working with the four hypotheses given above is still effective. If the evidence supports the inclusion of P_1 but not P_2, then typically $LR_{12/aa} < LR_{1a/aa}$ and $LR_{2a/aa} << 1$. In such a case, equation 3 is approximately $LR_{1a/aa}$. Even if $LR_{12/aa} > 1$, meaning that P_2 has been carried by P_1 into an inclusionary *LR* for H_{12} versus H_{aa}, the fact that $LR_{12/aa} < LR_{1a/aa}$ means that $LR_{1/\bar{1}}$ is approximately $LR_{1a/aa}$.

$LR_{1/\bar{1}}$ represents the support for the presence of donor 1 with or without the presence of donor 2. As such, it could be termed exhaustive within the context of the case. It is not, however, exhaustive in every sense, as there could be other propositions outside the set of four considered that may have a non-zero prior (for example the presence of donor 1, donor 2, and an unknown third person). We will therefore term $LR_{1/\bar{1}}$ the *LR given contextually exhaustive propositions* (contextually exhaustive LR for short). As always, the provision of additional information may necessitate re-evaluation of the findings and reconsideration of the assumptions.

Here we implement an equivalent to equation 3:

$$LR_{1/\bar{1}} = \frac{LR_{12/2a} + LR_{1a/aa} \big/ LR_{2a/aa}}{1 + 1 \big/ LR_{2a/aa}} \quad \text{(equation 4)}$$

Equation 4 has lower run time and is better estimated than equation 3; however, the behaviour of equation 4 is harder to visualise than equation 3.

It is possible to ensure a conservative $LR_{1/\bar{1}}$ using equations 3 or 4 if that is desired. This can be achieved in a number of ways, including:

1. For equation 3 this may be obtained by using conservative values for $LR_{12/aa}$ and $LR_{1a/aa}$ and a point estimate for $LR_{2a/aa}$.

2. For equation 4 this may be obtained by using conservative values for $LR_{12/2a}$ and $LR_{1a/aa}$ and a point estimate for $LR_{2a/aa}$.

1.5 P_1 or P_2 but not both

Using equation 3 it is straightforward to examine the situation where $LR_{1a/aa} = x$, $LR_{2a/aa} = y$, but $LR_{12/aa} = LR_{12/1a} = LR_{12/2a} = 0$. Equation 3 becomes $LR_{1/\bar{1}} = \frac{0+x}{y+1} \approx \frac{x}{y}$. If $x \approx y >> 1$, then $LR_{1/\bar{1}} \approx 1$. In such a case, the analysis correctly states that the evidence supports the proposition that P_1 and an unknown person or P_2 and an unknown person could be the sources of the DNA, but P_1 and P_2 cannot together be the donors. Further, if $x \approx y$, the evidence does not strongly support P_1 over P_2. We will discuss below the case where $x >> y$.

1.6 The effect of deviation from assumption 1 (i.e. prior weight of sub-proposition = 0.5)

Above we assumed that there was little information in I to inform the prior probability of the sub-proposition given that the primary proposition is true: $\Pr(H_{12} \mid H_1, I)$, $\Pr(H_{1a} \mid H_1, I)$ $\Pr(H_{2a} \mid H_{\bar{1}}, I)$, and $\Pr(H_{aa} \mid H_{\bar{1}}, I)$. There are two potential objections:

1. There may be case-relevant information available, for example P_1 and P_2 may have been seen together shortly before or after the crucial time, or
2. It may be inappropriate to model indifference of these prior weights either by a point value or more specifically as ½.

The LR is often linearly related to these prior weights and its behaviour is predictable. For example the equivalent of equation 3 retaining the prior weights is

$$LR_{1/\bar{1}} = \frac{LR_{12/aa}\frac{\Pr(H_{12} \mid H_1, I)}{\Pr(H_{aa} \mid H_{\bar{1}}, I)} + LR_{1a/aa}\frac{\Pr(H_{1a} \mid H_1, I)}{\Pr(H_{aa} \mid H_{\bar{1}}, I)}}{LR_{2a/aa}\frac{\Pr(H_{2a} \mid H_{\bar{1}}, I)}{\Pr(H_{aa} \mid H_{\bar{1}}, I)} + 1}$$

These prior weights were actually hidden in previous methods. For example, all of the proposition sets listed in section 1.2 effectively set the prior weights on the terms in the other set(s) to zero. The approach advocated here is more transparent and uses all the information.

1.7 Three-person formula

By extension, we could also consider the circumstance where an apparent three-person mixture has been recovered. Reference samples are available from three POI. Again, $LR_{1/\bar{1}}$ for P_1 in this case example can be assigned a number of ways. We offer two options below:

$$LR_{1|\bar{1}} = \frac{LR_{1aa/aaa} + LR_{2aa/aaa}LR_{12a/2aa} + LR_{3aa/aaa}LR_{13a/3aa} + LR_{2aa/aaa}LR_{23a/2aa}LR_{123/23a}}{1 + LR_{2aa/aaa} + LR_{3aa/aaa} + LR_{2aa/aaa}LR_{23a/2aa}} \quad \text{(equation 5)}$$

Or $$LR_{1|\bar{1}} = \frac{LR_{1aa/aaa} + LR_{2aa/aaa}LR_{12a/2aa} + LR_{3aa/aaa}LR_{13a/3aa} + LR_{3aa/aaa}LR_{23a/3aa}LR_{123/23a}}{1 + LR_{2aa/aaa} + LR_{3aa/aaa} + LR_{3aa/aaa}LR_{23a/3aa}} \quad \text{(equation 6).}$$

Equations (5) and (6) assume equal prior weights for the 8 propositions underlying the 7 *LR*s. The values assigned using these equations may differ slightly due to the variation induced by the estimation process.

1.8 More complex situations

Above we describe a relatively simple situation, a two-donor (or three-donor) mixture with two (or three) POI. Casework often presents much more complex situations. Examples would include an *N*-donor mixture when there are more than *N* POI, some of whom are related to each other. It may be necessary to consider unknown contributors under H_p as well. This situation can be aggravated by a lack of background information to assist in forming propositions. Unfortunately, this leads to a great many possible combinations of propositions, and exhaustive exploration is close to impossible.

Strategies for handling these challenging samples include careful sample choice to limit *N*, communication between scientists and law enforcement to ascertain the case-relevant background information *I*, and sensible selection to limit the number of POI being considered. As discussed in [7], these situations might be more suited for investigative purposes.

2.0 Method

To illustrate the added value of the use of contextually exhaustive propositions in casework, *LR*s assigned using different proposition sets were explored. A series of two- and three-donor GlobalFiler™ mixtures were selected from the PROVEDIt dataset [8]. A summary of these profiles is given in Table 1.

Table 1. Summary of profiles used. Profiles were selected from the PROVEDIt GlobalFiler™ 3500 15 s injection dataset.

Sample name	Number of contributors	Target mixture ratio	PCR DNA template amount (ng)
F02_RD14-0003-40_41-1;4-M3a-0.625GF-Q0.6_06.15sec	2	4:1	0.625
B05_RD14-0003-48_49-1;4-M2a-0.625GF-Q0.7_02.15sec	2	4:1	0.625
C04_RD14-0003-42_43-1;9-M2a-0.75GF-Q0.5_03.15sec	2	9:1	0.750
G07_RD14-0003-35_50-1;9-M2a-0.63GF-Q0.7_07.15sec	2	9:1	0.630
B01_RD14-0003-31_32-1;1-M1a-0.25GF-Q1.2_02.15sec	2	1:1	0.250
A08_RD14-0003-49_50_29-1;4;1-M3a-0.186GF-Q0.5_01.15sec.hid	3	4:1:1	0.186
B06_RD14-0003-46_47_48-1;1;1-M2a-0.375GF-Q0.4_02.15sec.hid	3	1:1:1	0.375

Each profile was analysed within GeneMapper® *ID-X* V1.5 using the following analytical thresholds: 6-FAM™ = 75 rfu, VIC™ = 100 rfu, NED™ = 60 rfu, TAZ™ = 80 rfu, SID™ = 100 rfu, LIZ™ = 60 rfu. Additional analysis settings can be provided by the authors upon request. Following analysis, the apparent number of contributors (NOC) was assigned for each profile using the maximum allele count method in conjunction with peak height information. For each profile examined, apparent NOC corresponded with the experimental design NOC. The profiles were then interpreted using the probabilistic genotyping software STRmix™ V2.7 [9, 10] using the parameters described in [11].

Illustration 1. True and simulated compatible pairs (sensitivity)

The aim of this experiment was to illustrate and demonstrate the value of the formulae produced (equations 2-6). We compared the *LR*s assigned using different proposition sets for compatible combinations of true and simulated donors. We assigned *LR*s as advocated within the ISFG guidelines [1], as in Buckleton et al. [7], and using contextually exhaustive propositions. This led to the calculation of:

$LR_{ia/aa}$ and $LR_{ij/ja}$ for the two-person mixtures, and $LR_{iaa/aaa}, LR_{ija/jaa}, LR_{ika/kaa}$, and $LR_{ijk,jka}$ for the three-person mixtures, where *i*, *j*, and *k* represent the POI under consideration. These *LR*s were assigned for the known donors to each mixture and additionally for a number of simulated donors that were created using the genotype weights from the unconditioned STRmix™ deconvolution. For each of the two-person major/minor mixtures listed in Table 1, three simulated minor donors were generated who were moderate to poor fits with the profile. For the unresolvable two-person mixture, three pairs of co-contributor profiles were prepared.

These pairs were made to have either the best fitting, moderate fitting, or poorest fitting genotype combination at each locus, where fit is defined using the probability of the profile given this genotype combination (i.e. the genotype weights as reported by STRmix™).

The NIST African American, Caucasian, and Hispanic allele frequencies [12] were used with $\theta = 0.01$. The point estimates of the *LR*s given sub-source propositions were used to calculate $LR_{1/\bar{1}}$ using equation 3 for the two-person mixtures or equation 5 for the three-person mixtures.

Illustration 2. Incompatible pairs of true and simulated true donors

For the unresolvable two-person mixture, each of the known donors was paired with one of the simulated donor profiles generated above. Within all pairings, $LR_{ia/aa}$ and $LR_{ja/aa} > 1$; however, $LR_{ij/aa} = 0$.

Illustration 3. False donor testing (specificity)

The unresolvable two-person mixture was digitally scaled down in height to 10% of its actual height in order to reduce the information content of the profile. This led to dropout of a number of alleles. Resulting peak heights varied from 77 to 284 rfu. The profile was interpreted using STRmix™ V2.7 using the settings described by Kelly et al. [11]. Ten

million (10^7) false donor profiles were simulated using the FBI Caucasian allele frequencies [13]. These were compared to the scaled profile and $LR_{ij/ja}$ and $LR_{ia/aa}$ were calculated using $\theta = 0$ and the FBI Caucasian allele frequencies within DBLR™ [14]. The $\log_{10}LR$ values were stored if ≥-100 for both calculations.

The peak heights of the three-person unbalanced mixture (3p 4-1-1) were reduced by half to increase the number of adventitious matches. This mixture was then searched against a database of 10,000 non-contributor profiles simulated using the NIST Caucasian allele frequencies. *LR*s were assigned for each database profile using the NIST Caucasian, NIST African American, and NIST Hispanic populations all with $\theta = 0.01$. The largest *LR* observed was in the order of 150 to 1000 depending on the allele frequencies used. This non-contributor profile (random 2046) aligned with contributor position 3 which is normally occupied by known donor Ref 29. This known contributor was replaced with the non-contributor profile, and the contextually exhaustive *LR* for the two remaining known donors (Refs 49 and 50) and the random non-contributor (random 2046) were calculated.

3.0 Results

3.1 Experiments regarding sensitivity

3.1.1 Two-person major/minor mixtures

In Figure 1 we plot $LR_{ia/aa}$ and $LR_{ij/ja}$ versus the contextually exhaustive *LR*, $LR_{i/\bar{i}}$, for the four two-person mixtures with major/minor contributors (Table 1). For each mixture, *LR*s were assigned for the true donors and for three compatible simulated contributors constructed as moderate to poor fits to the minor profile. For each comparison there are three *LR*s, one for each sub-population. Points below the dashed line at $x = y$ indicate that the contextually exhaustive *LR* was greater than $LR_{ia/aa}$ or $LR_{ij/ja}$. $LR_{ij/ja}$ is virtually equal to $LR_{i/\bar{i}}$ as indicated by the relevant data points falling on the dashed line. In a few cases $LR_{ia/aa}$ was much smaller than the contextually exhaustive *LR*. In our simulations the largest difference was $LR_{ia/aa} = 1$ and $LR_{i/\bar{i}} = 10{,}000$; this appears to be due to a genetic anomaly at locus D1S1656 within the DNA profile of PROVEDIt donor 41.

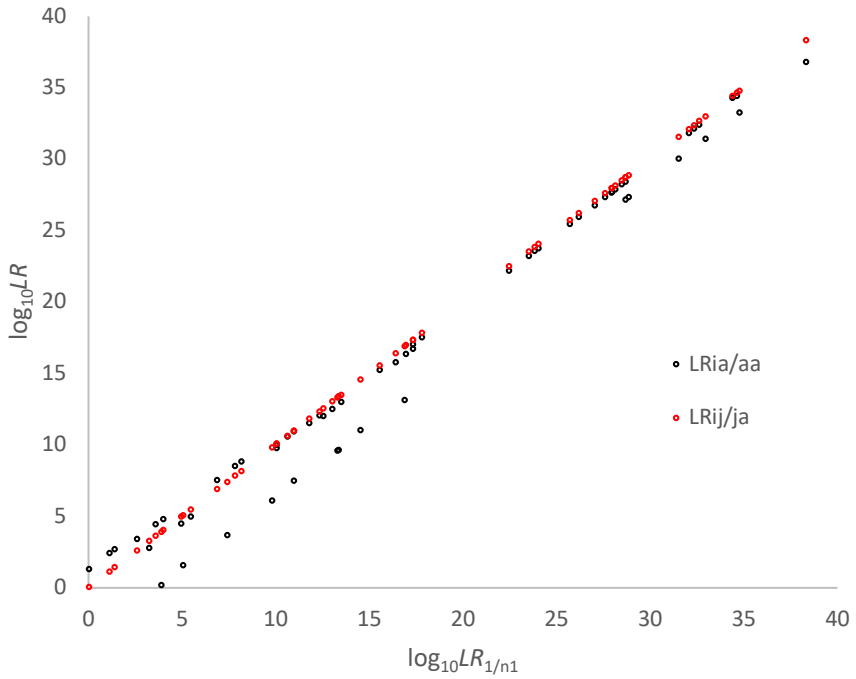


Figure 1. Plot of $LR_{ia/aa}$ and $LR_{ij/ja}$ versus $LR_{i/\bar{i}}$ (labelled $LR_{1/n1}$) for the major/minor two-person mixtures (approx. 4:1 and 9:1). *LR*s were assigned using allele frequency data for three sub-populations and have been plotted in $\log_{10}$ format. A dashed line at $x = y$ has been added to the plot to assist with interpretation. The data come in pairs, namely ($LR_{i/\bar{i}}$, $LR_{ia/aa}$) and ($LR_{i/\bar{i}}$, $LR_{ij/ja}$).

3.1.2 Two-person 1:1 mixture

In Figure 2 we plot $LR_{ia/aa}$ and $LR_{ij/ja}$ versus $LR_{i/\bar{i}}$ for the 1:1 two-person mixture for the four compatible pairs of POI (known donors and three pairs of simulated contributor profiles). In Figure 3 we plot $LR_{ia/aa}$ and $LR_{ij/ja}$ versus $LR_{i/\bar{i}}$ for the 1:1 two-person mixture using incompatible pairs of POI. Each pair consisted of one of the known donors and one of the simulated donor profiles, paired in such a manner that the two donors together did not explain the mixture. As expected, $LR_{ij/ja} = 0$ for all incompatible comparisons, and these exclusions are plotted as log(*LR*) = -40 in Figure 3. Consider, for example, the datum at (16.3, 20.5). For this datum $LR_{ij/ja} = 0$ because *i* and *j* are incompatible. $LR_{ia/aa} = 3.5\times10^{20}$, $\log_{10} LR_{ia/aa} = 20.5$, $LR_{ja/aa} = 1.9\times10^{4}$, $\log_{10} LR_{ja/aa} = 4.3$, and hence (using equation 4) for contributor *i*

$$\log_{10} LR_{i/\bar{i}} = \log_{10} \frac{0 + 3.5\times10^{20} \,/\, 1.9\times10^{4}}{1 + 1 \,/\, 1.9\times10^{4}} = 16.3$$

.

For contributor *j*, $LR_{ij/ia} = 0$ because *i* and *j* are incompatible, and

$$\log_{10} LR_{j/\bar{j}} = \log_{10} \frac{0 + 1.9\times10^{4} / 3.5\times10^{20}}{1 + 1 / 3.5\times10^{20}} = -16.3$$

(note $\log_{10} LR_{j/\bar{j}}$ does not exactly equal $-\log_{10} LR_{i/\bar{i}}$, as there is a difference in the 5th significant figure). This is the datum at (-16.3, 4.3).

Where $LR_{1a/aa} >> LR_{2a/aa}$ (for example, where one of the known donors was paired with a poor-fitting simulated donor), the contextually exhaustive *LR* for POI 1 gave relatively strong support for inclusion. The corresponding $LR_{i/\bar{i}}$ for POI 2 gave relatively strong support for exclusion. In contrast, where $LR_{1a/aa} \approx LR_{2a/aa,}$ values assigned for $LR_{i/\bar{i}}$ were spread around $\log_{10}(LR_{i/\bar{i}}) = 0$.

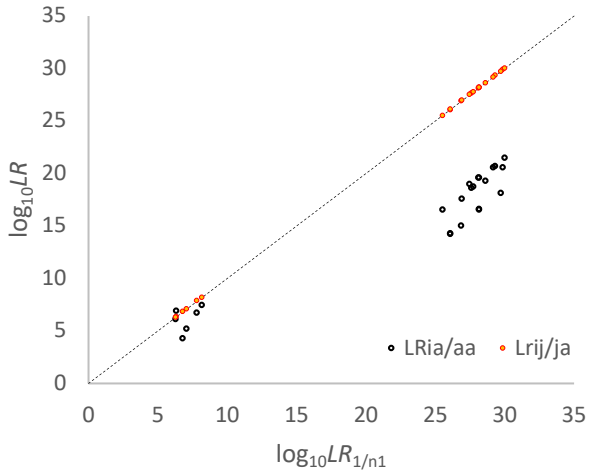
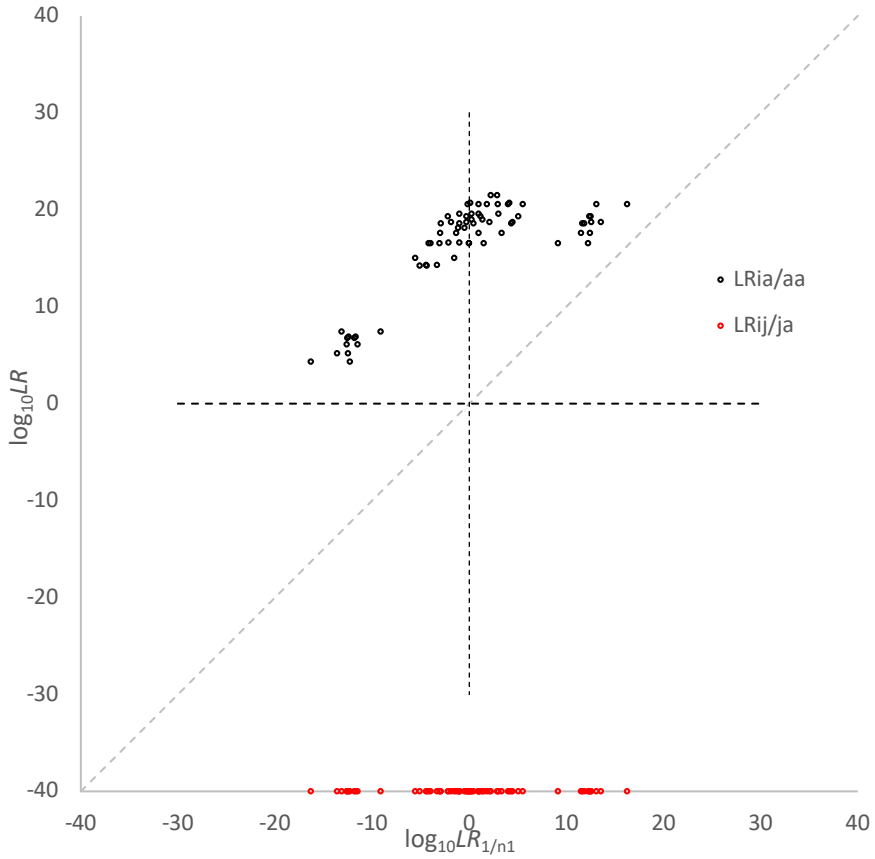


Figure 2. Plot of $LR_{ia/aa}$ and $LR_{ij/ja}$ versus $LR_{i/\bar{i}}$ (labelled $LR_{1/n1}$) for the 1:1 two-person mixture for the four compatible pairs of POI (known donors and three pairs of simulated contributor profiles). *LR*s were assigned using allele frequency data for three sub-populations with $\theta = 0.01$ and have been plotted in $\log_{10}$ format. A dashed line at $x = y$ has been added to the plot to assist with interpretation.



355

356 Figure 3. Plot of $LR_{ia/aa}$ and $LR_{ij/ja}$ versus $LR_{i/\bar{i}}$ (labelled $LR_{1/n1}$) for the 1:1 two-person
357 mixture using incompatible pairs of POI. Each pair consisted of one of the known donors as
358 well as one of the simulated donor profiles. *LR*s have been plotted in $\log_{10}$ format. A dashed
359 line at $x = y$ and vertical and horizontal lines at $\log_{10}(LR) = 0$ have been added to the plot to
360 assist with interpretation. Exclusions ($LR = 0$) are plotted as $\log_{10}(LR) = -40$.

361 *3.1.3 Three-person mixtures*

362 In Figure 4 we plot $LR_{iaa/aaa}$ and $LR_{ijk/jka}$ versus $LR_{i/\bar{i}}$ for the two three-person mixtures.

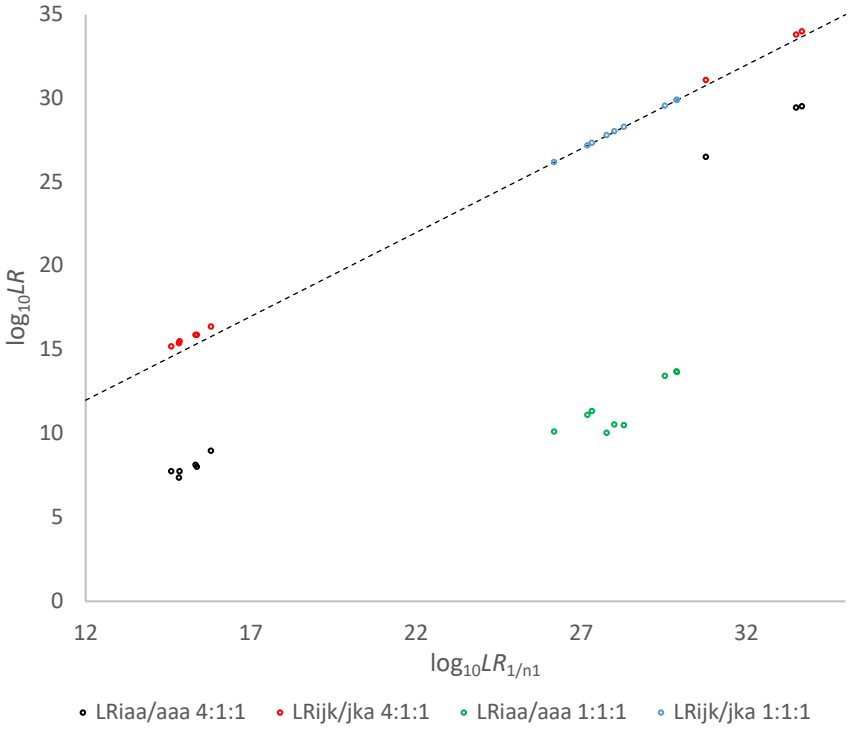


Figure 4. Plot of $LR_{iaa/aaa}$ and $LR_{ijk/jka}$ versus $LR_{i/\bar{i}}$ (labelled $LR_{1/n1}$) for the three-person mixtures (approx. 1:1:1 and 4:1:1). *LR*s were assigned using allele frequency data for three sub-populations and have been plotted in $\log_{10}$ format. A dashed line at $x = y$ has been added to the plot to assist with interpretation.

3.2 Experiments regarding specificity

3.2.1 False donor results

The distribution of $\log_{10}LR_{ia/aa}$ for ten million (10^7) non-contributor comparisons where $\log_{10}LR \geq -100$ (N=3437) is plotted in Figure 5. There were 67 $\log_{10}LR_{ia/aa}$ values > 0.

There were no $\log_{10}LR_{ij/ja}$ values ≥ -100.

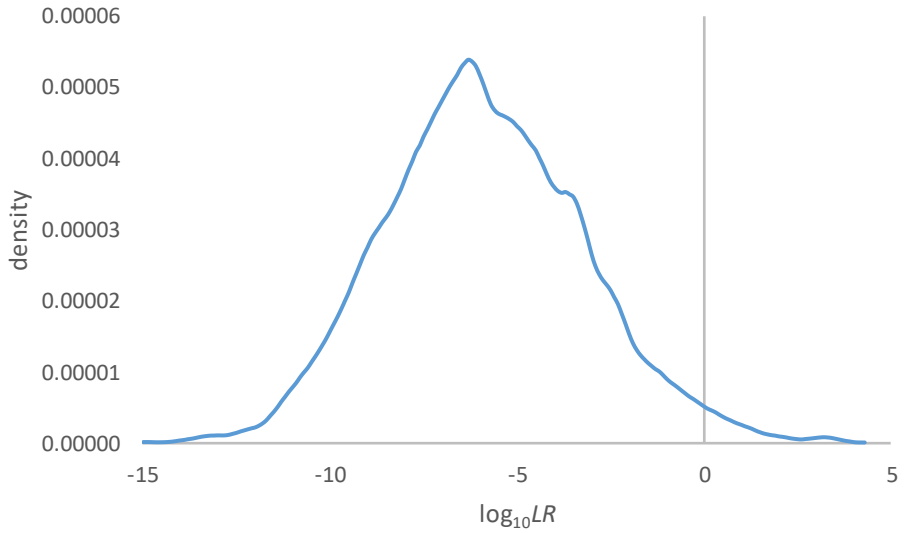


Figure 5. A plot of density versus $\log_{10}LR_{ia/aa}$ for the 10,000,000 non-contributor tests. There are 3437 results represented in the plot, with the remaining non-contributor profiles giving $\log_{10}LR_{ia/aa} < -100$.

3.2.2 False donor results: true donor and adventitious match

As previously stated, an adventitious match to the 3-person 4:1:1 mixture (with peak heights reduced by half) was identified after searching the STRmix™ deconvolution against a database of 10,000 simulated non-contributor profiles. The *LR* assigned for the non-contributor (random 2046) ranged from approximately 150 to 1000 depending on the choice of population used. The non-contributor profile aligned best with contributor position 3, which is normally occupied by known donor Ref 29. Below, we provide a plot of $LR_{iaa/aaa}$ and $LR_{ijk/jka}$ versus $LR_{i/\bar{i}}$ for the two remaining known donors (Refs 49 and 50) and random 2046 (i.e. replacing Ref 29 with random donor 2046). Overall, the contextually exhaustive *LR*s gave strong support for inclusion for the two known donors and strong support for exclusion of the non-contributor. In contrast, $LR_{iaa/aaa}$ falsely supported the inclusion of random 2046 whilst $LR_{ijk/jka}$ falsely excluded the two known donors.

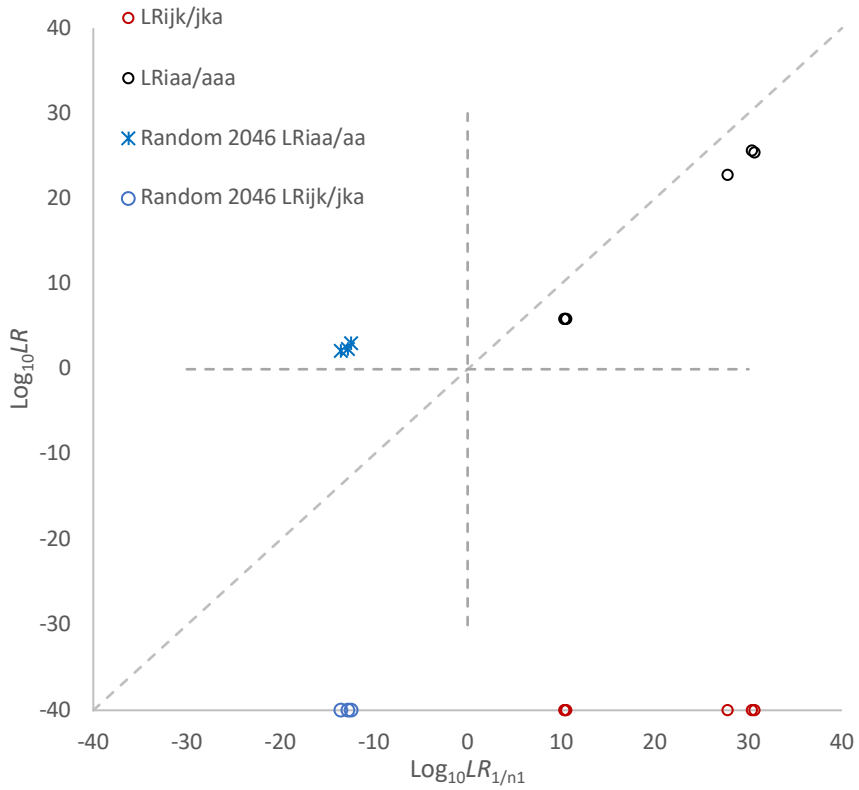
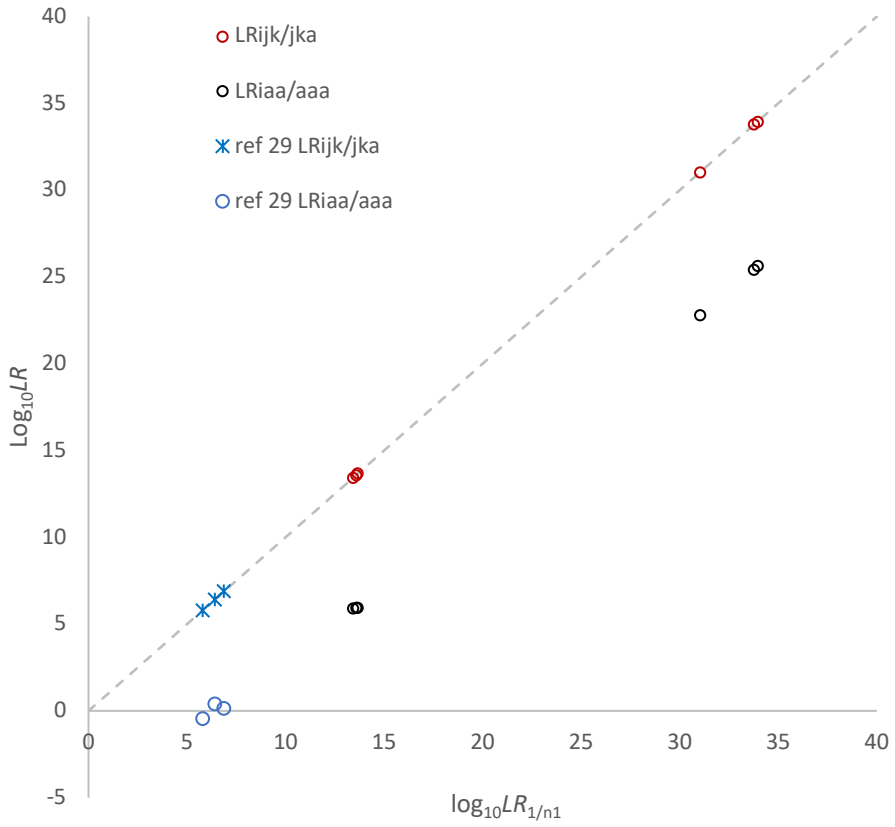


Figure 6: Plot of $LR_{iaa/aaa}$ and $LR_{ijk/jka}$ versus $LR_{i/\bar{i}}$ (labelled $LR_{1/n1}$) for the three-person mixture (approx. 4:1:1) with peak heights reduced. *LR*s were assigned for two of the known donors as well as a non-contributor profile that gave rise to an adventitious match when $LR_{iaa/aaa}$ was assigned. *LR*s were assigned using allele frequency data for three sub-populations and have been plotted in $\log_{10}$ format. Exclusions ($LR = 0$) have been plotted as $\log(LR) = -40$. A dashed line at $x = y$ and vertical and horizontal lines at $\log_{10}(LR) = 0$ have been added to the plot to assist with interpretation.

As a further investigation, Ref 29 was reinstated and $LR_{i/\bar{i}}$ was assigned for the three known donors (Refs 29, 49, and 50). The contextually exhaustive *LR*s assigned are illustrated in Figure 7 below along with $LR_{iaa/aaa}$ and $LR_{ijk/jka}$. As in our previous experiments, $LR_{i/\bar{i}}$ aligned very closely with $LR_{ijk/jka}$. Of interest, the contextually exhaustive *LR*s for Ref 29 correctly supported inclusion with $\log_{10} LR_{i/\bar{i}}$ values ranging from 5.79 to 6.88 depending on the population used. In contrast, $LR_{iaa/aaa}$ produced values that were close to neutral (NIST Caucasian: $\log_{10}LR = -0.44$, NIST African American: $\log_{10}LR = 0.15$, NIST Hispanic: $\log_{10}LR = 0.39$).



405

406 Figure 7: Plot of $LR_{iaa/aaa}$ and $LR_{ijk/jka}$ versus $LR_{i/\bar{i}}$ (labelled $LR_{1/n1}$) for the three-person
407 mixture (approx. 4:1:1) with peak heights reduced. *LR*s were assigned for the three known
408 donors. *LR*s were assigned using allele frequency data for three sub-populations and have
409 been plotted in $\log_{10}$ format. A dashed line at $x = y$ has been added to the plot to assist with
410 interpretation.

411 **4.0 Discussion**

412 For the true donors and compatible simulated donors to the two- and three-person mixtures,
413 $LR_{ij/ja}$ or $LR_{ijk/jka}$ is close to $LR_{i/\bar{i}}$ whether $LR_{ij/ja}$ (or $LR_{ijk/jka}$) is near the top of the examined
414 range (good fit to the profile) or the bottom (poor to moderate fit to the profile). This is the
415 expected result from examination of equations 3, 4, and 5. If $LR_{i/\bar{i}}$ is treated as the gold
416 standard, as we suggest, then $LR_{ij/ja}$ is a good approximation in most, but not all, cases.

417 For the incompatible pairs of donors examined for the 1:1 mixture,
418 $0 \approx LR_{ij/ja} < LR_{i/\bar{i}} \approx LR_{ia/ja} < LR_{ia/aa}$. We can intuitively understand why this result is
419 expected. The two POI separately give an inclusionary *LR*, but this *LR* overlooks the fact
420 that, for each mixture, there is an alternative POI who is a reasonable fit to the profile but
421 incompatible with the first POI. In actual casework most laboratories do test the two POI
422 together even if they intend to report the two $LR_{ia/aa}$. If the two are incompatible, leading to
423 an exclusionary *LR*, this result should be reported. However, it is then generally left to non-
424 scientists to infer what these apparently conflicting results mean.

Because we have selected incompatible pairs for this experiment, $LR_{ij/ja}= 0$. This is also potentially a misrepresentation of the evidence since both POI should give an inclusionary *LR* and the value of $LR_{ij/ja}= 0$ might imply exclusion. $LR_{i/\bar{i}}$ correctly balances these factors but we suggest that it cannot be reported without further explanation. We give some options in the appendix.

The power of DNA analysis and probabilistic genotyping is demonstrated by the false donor tests. It was necessary to lower the peak heights of the profile to obtain any *LR*s > 1 for the non-contributors, even after comparison with ten million non-contributor profiles. It is known that conditioning on a known contributor improves the power of the analysis to differentiate true from false donors for the remaining contributors [15]. The unconditioned $LR_{ia/aa}$ gave few *LR* values > 1 for non-contributors, whereas the conditioned $LR_{ij/ja}$ gave none out of ten million comparisons. From this we would infer that often, for false donors, $LR_{ij/ja} < LR_{i/\bar{i}} < LR_{ia/aa}$. Since, in casework, it is usually unknown whether we are comparing with a true or a false donor, we strongly suggest that the background information be gathered and considered. If conditioning is well justified then $LR_{ij/ja}$ should be used. If conditioning is ambiguous then the best compromise is probably $LR_{i/\bar{i}}$.

The experiments done with true donors and an adventitiously matching candidate demonstrate that including relevant information and using $LR_{i/\bar{i}}$ offers better discrimination of the propositions of interest: the true candidates having a larger *LR* and the adventitious candidate being excluded. The use of the $LR_{i/\bar{i}}$ has been shown to be more meaningful and allows better sensitivity and specificity.

Conclusions

When a mixture profile is to be compared to several POI, the propositions being considered should account for all the potential contributors. This is accomplished by selecting a set of propositions that are exhaustive within the context of the case at hand. From a theoretical and experimental point of view we conclude that the *LR* assigned from these contextually exhaustive propositions, $LR_{i/\bar{i}}$, is a more meaningful statistic to provide to the fact finder for either true donors or non-contributors.

Conflict of interest

[Removed for refereeing]

Acknowledgements

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Appendix

We make no claim to knowing what reporting styles are preferred. The following are two options that might operate as starting positions. Both assume that a DNA mixture from three contributors is compared to P_1 and P_2. "Unknown" people are assumed to also be unrelated.

Option 1. The DNA results are $LR_{1a/aa}$ times more likely if P_1 is a donor than if he is not. The DNA results are $LR_{2a/aa}$ times more likely if P_2 is a donor than if he is not. This is [verbal qualifier] support for the proposition that P_1 is a donor rather than not and [verbal qualifier] support that P_2 is a donor rather than not. However, both P_1 and P_2 cannot be donors together. Details of this analysis are provided in Table A1.

Table A1. The propositions and *LR*s for option 1

Proposition	*Alternative*	*LR*
The donors to the evidence are P_1 and two unknown people	The donors to the evidence are three unknown people	$LR_{1a/aa}$
The donors to the evidence are P_2 and two unknown people	The donors to the evidence are three unknown people	$LR_{2a/aa}$
The donors to the evidence are P_1, P_2, and one unknown person	The donors to the evidence are three unknown people	0

Option 2. The following propositions were considered:

(1) the DNA mixture comes from P_1, P_2, and an unknown person

(2) the DNA mixture comes from P_1 and two unknown people (but not P_2)

(3) the DNA mixture comes from P_2 and two unknown people (but not P_1)

(4) the DNA mixture comes from three unknown people.

We will summarize *(1)* and *(2)* as P_1 is a contributor to the mixture, and *(3)* and *(4)* as P_1 is not a contributor. We have considered that *(1)* and *(2)* are equally probable if P_1 is a contributor. Similarly, (3) and (4) were assigned the same probability if P_1 is not a contributor. The same reasoning applies for P_2.

The comparison of the DNA profiles shows that together P_1 and P_2 cannot be contributors to the mixture.

For P_1, the DNA results are on the order of a billion times more likely if he is a contributor to the mixture than if he is not.

For P_2, the DNA results are on the order of 10,000 times more likely if he is **not** a contributor to the mixture than if he is. This result strongly supports exclusion.