SDPD Forensic Science Section – Forensic Biology Unit **July 26, 2016**

Validation of the STRmix^{TM} Software – Addendum

Likelihood ratio - inconclusive range (4 Person Mixture Hp true tests)

Purpose

After approximately 6-months of casework experience it was determined that a higher likelihood ratio threshold (currently 10^{-6}) below which a person of interest could be excluded outright, without the use of a verbal predicate, would simplify communicating the results to the end user and the information would become more digestible to them. This should result in less confusion and more understanding of our conclusions with regard to LR values favoring exclusion.

The goal of this study was to obtain additional data to evaluate a likelihood ratio level below which we could confidently exclude non-contributors without excluding true contributors. The previous validation studies that examined specificity and sensitivity were used to inform the inconclusive range used by the laboratory. The inconclusive range was determined to be between LR values of 0.01 and 100 for single source through 4-person mixtures. Less complex and higher template samples generally resulted in clear inclusions and inclusions. As mixtures became more complex (more contributors) or contributor template levels decreased, more non-contributor LR values approached 1 (with some slightly above) and some true contributor LR values obtained were less than 1. This study re-evaluated the previous comparisons of validation mixtures to true contributors and non contributions (see the STRmix Specificity and Sensitivity Study write-up). Furthermore, based on previous validation data, additional 4-person mixtures were selected for this study due to their inherent complexity and possibility of obtaining low LRs for true contributors. These 4-person mixtures, that had not been analyzed previously with STRmix) were deconvoluted and compared to the validation database to determine the lowest likelihood ratio value obtained for true contributors. These additional 46 samples included high, mid, and low level mixtures with a range of contributor proportions.

Materials and Methods

The initial sensitivity and specificity data was re-evaluated looking specifically for information on the lowest LR values for true contributors. This previous data set consisted of ten 2-person mixtures, seventeen 3-person mixtures, and sixteen 4-person mixtures. Database search LRs were calculated by searching a validation database of 76 people.

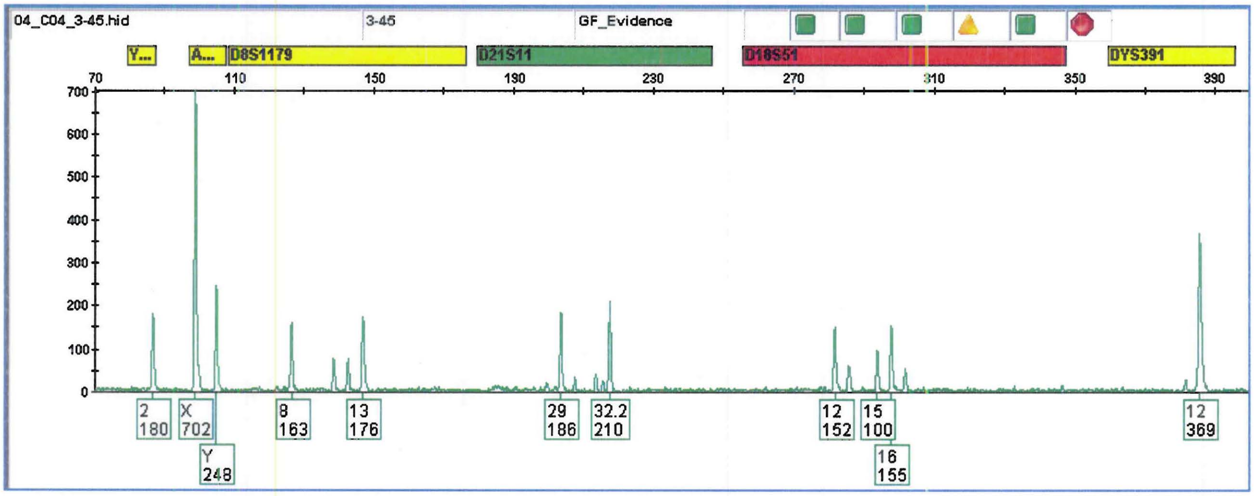
46 4-person mixtures had previously been created by three different analysts as part of the GlobalFiler/3500/STRmix validation. All 46 of these mixtures were evaluated with STRmix (casework settings) as 4 person mixtures and then compared against the validation database. Any samples from true contributors in the validation database the obtained database search LR

values were recalculated using the LR from previous analysis function to incorporate theta into the likelihood ratio.

Results and Discussion

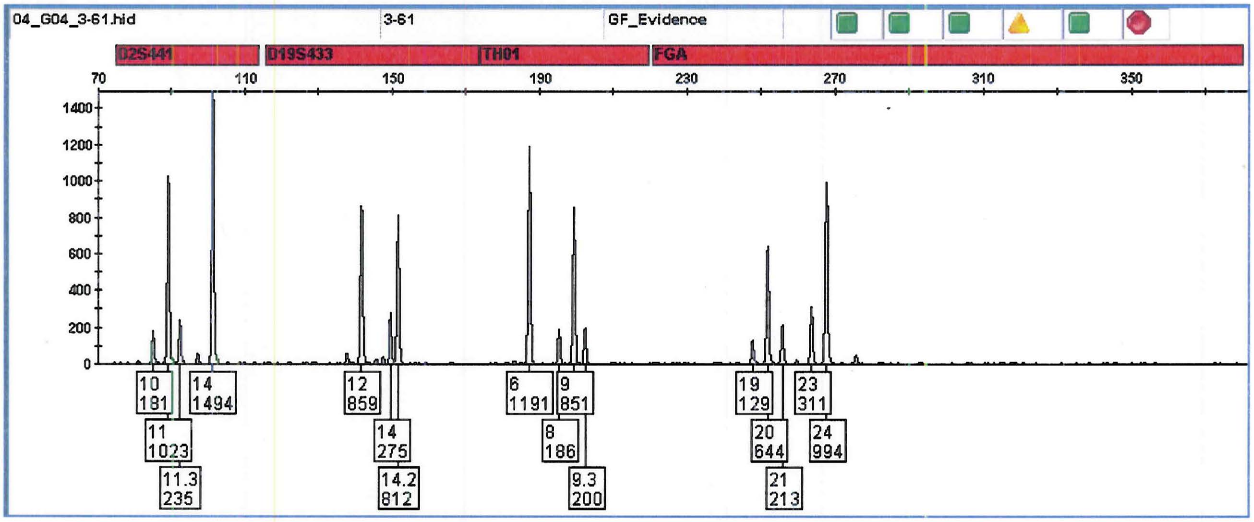
The previous sensitivity and specificity data comprising 43 mixture samples where 10,260 comparisons were made to the validation database. The only mixtures where false exclusions ($<10^{-2}$) were obtained were all low level mixtures that contained extensive drop-out. These mixtures all had average peak heights below 600rfu, which is the point where drop-out becomes likely. The LR values indicating false exclusions were 2×10^{-4} for mixture 3-45 and 1.5×10^{-3} for mixture 3-61. In both instances the false exclusions were obtained for the lowest of the three contributors in the mixtures.

Mixture 3-45 is presented below. This mixture is from 3 contributors although no locus had more than 4 detected alleles. A couple of loci (including D18S51 below) had indications of the third contributor. The true contributor that had a negative log likelihood ratio suggesting exclusion (2×10^{-4}) was missing 20 alleles from the detected alleles in the mixture, including complete genotype drop-out at several loci.



Given the number of missing alleles, it is not surprising that this contributor had a LR indicative of exclusion. In addition, it is likely this sample would not have been interpreted by analysts in the lab based on the fact that this 3-person mixture (as indicated by D18S51) had no loci with a detected 5th allele.

Mixture 3-61 is presented below. This mixture is from 3 contributors and only one locus had 5 detected alleles. The true contributor that had a negative log likelihood ratio suggesting exclusion (1.5×10^{-3}) was either partially or fully dropping out at 10 loci and completely masked at 5 loci. Of the remaining 6 loci, this contributor was fully represented at only 2 loci, while being partially masked at 4.



All indications are that this sample, or samples like this, would be interpreted and analyzed with STRmix if they had been observed in casework. Given that the LR values for the other two true contributors were 10^{13} and 10^{31}, and no non-contributors received LR values greater than one, the STRmix deconvolution meets expectations. That one of the true contributors received an exclusionary LR value is not surprising given the low level of the results and the limited information regarding this contributor.

In examining the results from the newly analyzed mixtures, LR values were generated by searching the validation database of 76 individuals (13,984 comparisons) which include the 4 known contributors in each mixture. Table 1 lists the database search LR values of the 4 known contributors to each mixture. This table demonstrates strong support for inclusion of all contributors for most of the mixtures. The sample IDs highlighted in red indicate samples with a high amount of DNA, and sample IDs with orange and blue highlights indicate samples with medium and low amounts of DNA, respectively. Three of the samples with a low DNA amount had inconclusive database search LR values for at least one contributor (highlighted in green). This is not uncommon for samples with low template contributors exhibiting drop-out and masking from higher template contributors.

In most cases, low-level contributors were included with very strong LR values, but in some instances, due to contributor genotype ambiguity, some LR values obtained were in the inconclusive range. Three mixtures: 4-45, 4-52, and 4-56 (all low template level samples) resulted in database search LR values for contributors in the inconclusive range. The LR values ranged from 9.1 to as low as 0.50. A significant amount of the non-contributors for 4-45 were also inconclusive, although none had LR values greater than 100. This result is not unexpected given the very low amount of DNA for the four contributors in the sample.

The true contributors whose database search LR values were in the inconclusive range were recalculated using the Balding-Nichols adjustments incorporated (LR from previous analysis as opposed to the database search LR) to determine what a reported LR would be for the true contributors. For sample 4-45, the reported LRs for the true contributors would have been 2, 1.3, and 63, which are all still within the current inconclusive range of 0.01 to 100. For sample 4-52, the reported LR for the lowest-level true contributor would be 1.35, which is also within the inconclusive range. For sample 4-52, the reported LR for the lowest-level true contributor would be 3.3, which is again also within the inconclusive range.

Although, not pertinent to determining an exclusionary threshold, for most of the mixtures, known non-contributors had an LR of 0 (exclusionary). Some samples had LRs supporting exclusion for non-contributors for the low template samples. Regarding LRs for the known non-contributors, the highest database search LR for a non-contributor was 1.2 for sample 4-56 (a low template sample), which is inconclusive. This non-contributor had a point LR of 0.78.

Table 1- Likelihood ratios for the 4 known contributors in each mixture using the 76 person database search

4-1	4-2	4-4	4-5	4-6	4-8	4-9	4-10
6.60E+11	1.70E+10	1.10E+27	5.30E+22	1.10E+20	7.60E+28	1.00E+21	1.00E+20
1.30E+09	2.10E+13	5.30E+17	1.50E+24	2.00E+17	7.60E+10	1.40E+14	1.40E+17
2.90E+07	4.00E+17	1.00E+09	2.50E+17	1.90E+10	7.80E+20	3.70E+09	1.20E+13
1.50E+08	5.70E+14	2.50E+10	2.80E+15	2.20E+14	4.90E+19	2.90E+14	1.40E+21
4-12	4-13	4-16	4-17	4-20	4-21	4-24	4-25
6.00E+23	2.70E+15	1.10E+22	2.00E+26	2.60E+16	6.70E+11	1.60E+09	2.60E+25
1.70E+27	8.50E+14	2.80E+23	1.80E+18	3.10E+20	2.90E+15	4.40E+11	6.70E+12
1.90E+20	2.50E+16	1.80E+07	6.20E+21	3.40E+26	5.90E+10	3.40E+08	2.30E+09
1.30E+13	2.00E+20	1.90E+14	1.40E+20	2.00E+27	1.90E+17	1.60E+14	1.10E+14
4-26	4-28	4-29	4-30	4-32	4-33	4-34	4-36
4.80E+26	5.30E+23	2.10E+31	8.00E+28	1.40E+18	1.70E+16	5.00E+23	1.50E+23
3.00E+16	7.50E+07	1.80E+14	7.30E+14	3.60E+17	1.30E+19	2.00E+24	1.40E+26
1.10E+12	2.30E+15	9.00E+11	9.29E+02	1.80E+13	1.00E+31	3.50E+17	1.20E+14
3.50E+11	5.40E+12	4.00E+10	1.80E+18	6.80E+13	9.20E+10	1.50E+08	4.50E+11
4-37	4-38	4-40	4-41	4-42	4-44	4-45	4-46
1.20E+26	7.80E+21	3.70E+11	1.40E+17	6.30E+11	3.60E+12	1.20E+00	2.50E+07
4.50E+23	7.90E+22	2.70E+16	4.00E+20	1.00E+25	9.80E+09	1.20E+10	3.40E+10
1.40E+12	3.10E+13	5.80E+17	9.90E+11	1.40E+23	4.70E+11	2.80E+00	2.20E+07
3.60E+11	3.09E+06	3.50E+06	4.10E+13	5.00E+14	3.00E+11	3.36E+02	2.70E+18
4-48	4-49	4-50	4-52	4-54	4-56	4-57	4-58
6.90E+27	1.60E+24	3.50E+21	8.40E+28	8.40E+13	2.40E+27	4.80E+08	2.80E+16
1.50E+19	1.10E+15	1.00E+09	1.30E+15	2.02E+04	1.80E+30	1.30E+09	1.60E+22
7.70E+07	6.50E+13	8.90E+09	5.00E-01	4.30E+15	4.60E+20	3.50E+12	7.30E+09
2.50E+11	6.10E+11	8.50E+08	7.50E+17	1.10E+10	9.10E+00	2.50E+09	1.20E+07
4-60	4-61	4-62	4-64	4-65	4-66		
4.60E+17	6.00E+22	1.10E+12	7.80E+14	5.00E+14	1.30E+10		
2.70E+19	1.70E+18	8.10E+14	4.10E+15	1.10E+10	7.90E+12		
5.30E+09	1.90E+17	2.10E+17	1.20E+08	6.50E+11	1.50E+13		
8.00E+07	1.10E+14	3.70E+10	1.50E+10	1.20E+09	3.50E+12		

Conclusion

The data generated from the 46 additional 4-person mixtures through STRmix and searching the mixtures against the validation database of 76 individuals further supports our current inconclusive range.

With regards to LR values below which we can feel confident excluding individuals, the majority of true contributors received calculated LR values more than 10^{-2}. Only two true contributors had LR values that were below 10^{-2}. These were in the original specificity and sensitivity samples and were both from 3-person mixtures. Both instances involved contributors to low level mixtures that had numerous instances of allelic drop-out and allele sharing with other contributors. Given the circumstances surrounding these true contributors receiving exclusionary LR values, it seems appropriate to use 10^{-2} as a threshold below which exclusions could be supported without the use of verbal predicate.

Given these results the new verbal scale would be as follows:

Likelihood Ratio	Verbal Wording
>10,000	Strong support
1000-10,000	Moderate support
100-1000	Limited support
0.01 -100	Inconclusive
$<10^{-2}$ - 0	Excluded

8/12/16

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