

**The Zoom Study:** Additional Guidelines for Interpretation of Mixtures and Low Level Data Using GlobalFiler<sup>™</sup> on the 3500/3500xL and/or STRmix<sup>™</sup> 2.4

# The Zoom Study:

# Additional Guidelines for Interpretation of Mixtures and Low Level Data Using GlobalFiler™ on the 3500/3500xL and/or STRmix™ 2.4

This study has been reviewed and approved by:

Stephanie Hickey, Acting FBU Validation Technical Leader

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Additional assistance for this study was provided by Kathryn Carte, FBU Intern.

Edits made to original validation on January 14, 2022. See page 22 for details and approval. 01/19/2022 CJ

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#### I. Introduction

In 2017, an entirely new set of casework procedures (Amplification Cutoff, GlobalFiler™, 3500/3500xL, GeneMapper® *ID-X* 1.5, and STRmix™ 2.4.06) were validated and implemented at the Forensic Biology Unit (FBU) of the District of Columbia Department of Forensic Sciences (DC DFS). While the validations were complete and provided high quality data for standard operating procedures (SOPs) and analyst training, further experience in the interpretation of casework GlobalFiler™ data has suggested that a supplemental study may be useful in providing additional guidance for data interpretation. The study below will "zoom in" on low level DNA profiles, contributors, and overall mixtures to provide analysts with possible indicators of limited data during both the initial qualitative interpretation of a profile and/or subsequent analyst review of the STRmix™ deconvolution of a profile. The indicators being evaluated will include: average peak height, DNA amount, number of unique alleles, and percent contributor.

#### II. Materials, Methods, and Data Analysis

Data was gathered from the previous validation of STRmix<sup>™</sup> 2.4 for forty-eight low-level single source samples (approximately 250 pg or less), thirty 2-person mixture samples, twenty 3-person mixture samples, twenty 4-person mixture samples, and twenty 5-person mixture samples. DNA amount and percent contributor were gathered from the previously run STRmix<sup>™</sup> Advanced Reports. If a sample was not part of the previous validation, it was run in a performance-checked copy of the software and the data was recorded. All samples were run using the "*LR* from previous" feature to ensure data was associated with the appropriate contributor.

Additionally, profiles were evaluated to determine the number of unique (unshared) alleles that were obtained. For single source profiles, the number of alleles and the number of loci with results were counted and recorded. For 2-person mixture samples and 3-person mixture samples, the number of unique minor alleles were counted and recorded. For 4-person mixture samples and 5-person mixture samples, all unique alleles for each contributor were counted and recorded, regardless of contributor level, due to the complexity of higher order mixtures. Average peak height (APH) was calculated from the unique alleles. For non-contributors, the lowest level known contributor's APH was used for all plots. No data was gathered or recorded for Y indel, Amelogenin, or DYS391.

For single source and 2-person mixtures, likelihood ratios were recorded as the lowest likelihood ratio of the four population groups tested and included Highest Posterior Density (HPD). Due to data availability, likelihood ratios for all 3-, 4-, and 5-person mixtures were recorded as the lowest likelihood ratio of the four population groups tested and did not include HPD.

The following terms were used throughout this document and are defined below:

- True inclusion: A known contributor which produced a likelihood ratio > 1
- True exclusion: A non-contributor which produced a likelihood ratio < 1
- False inclusion: A non-contributor which produced a likelihood ratio > 1
- False exclusion: A known contributor which produced a likelihood ratio < 1



#### III. Results



**Figure III-A1**: Log(*LR*) versus number of alleles for low-level single source samples (approximately 250 pg or less)

As expected, the likelihood ratio (*LR*) increased as the number of alleles detected increased in single source samples (see Figure III-A1). The laboratory currently follows the Scientific Working Group on DNA Analysis Methods' (SWGDAM) recommendations for reporting genotyping results as likelihood ratios in which an *LR* of one is reported as "uninformative" and an inclusion with an *LR* between 1 and 100 is reported as "limited support". This verbal scale is in agreement with the data obtained in the validation which demonstrated overlap in likelihood ratios for low true inclusions and high false exclusions at *LR*s between 1 and 100. Based on the data from this study, samples with five alleles or more produced true *LR*s greater than 100, indicated as moderate support on the recommended verbal scale.



**Figure III-A2**: Log(*LR*) versus number of loci for low-level single source samples (approximately 250 pg or less)



Similarly, the *LR* increased as the number of loci with results increased in single source samples (see Figure III-A2). Based on this data, samples with results at five loci or more produced true *LR*s greater than 100.



**Figure III-A3**: Log(*LR*) versus APH (in RFU) for low-level single source samples (approximately 250 pg or less)



**Figure III-A4**: Log(*LR*) versus DNA amount for low-level single source samples (approximately 250 pg or less)

Figures III-A3 and III-A4 demonstrate that the *LR* increased as the average peak height and DNA amount increased. Average peak heights of greater than 122 RFU consistently produced an *LR* greater than 100. While DNA amounts greater than 230 consistently produced *LR*s greater than 100, the *LR*s varied significantly at DNA amounts from 150 to 230. This is most likely attributed to STRmix<sup>TM</sup> considering dropout and stochastic variation when calculating DNA amount. One sample with a DNA amount of 387 produced an *LR* less than 100. This sample's DNA amount was based on only three total alleles (the

![](_page_4_Picture_0.jpeg)

![](_page_4_Figure_2.jpeg)

minimum number of acceptable alleles for the software to reliably operate) and will be considered an outlier.

**Figure III-A5**: APH (in RFU) versus DNA amount for low-level single source samples (approximately 250 pg or less)

Figure III-A5 shows the variability between average peak height and DNA amount for low-level single source samples. Based on this information, average peak height may be a better indicator of the suitability of a low-level single source profile than DNA amount, especially when there are a low number of alleles. For example, the previously identified outlier with only three alleles and a DNA amount of 387 had an average peak height of 100 RFU.

# B. 2-person mixture samples

The plots below (Figures III-B1 to III-B4) are "zooms" of the lower-level contributors from the 2-person mixture samples to provide a more in-depth evaluation of the area where false inclusions/exclusions intersect. As expected, and demonstrated in the previous validation, higher-level contributors produce fully concordant inclusions/exclusions. It is important to note that each plot also demonstrates a significant number of full exclusions (Log(LR) < -5 and Log(LR) = 0) at low levels that are not shown. Full versions of each plot are available in the electronic data.

![](_page_5_Picture_0.jpeg)

![](_page_5_Figure_2.jpeg)

![](_page_5_Figure_3.jpeg)

False inclusions were obtained for the 2-person mixtures when the average peak height of the lower-level contributor was 128 RFU or less (see Figure III-B1). Two of those false inclusions, with average peak heights of 102 RFU, had *LR*s greater than 100 and one true inclusion had an *LR* less than 100 (limited support). One false exclusion (not shown) was obtained at 228 RFU due to an elevated stutter allele.

![](_page_5_Figure_5.jpeg)

**Figure III-B2**: Zoomed-in plot of Log(*LR*) versus DNA amount of the minor contributor for 2-person mixtures

False inclusions were obtained for the 2-person mixtures when the DNA amounts of the lower-level contributor was 37 or less (see Figure III-B2). At a DNA amount of 25, two false inclusions had *LR*s greater

![](_page_6_Picture_0.jpeg)

![](_page_6_Picture_1.jpeg)

than 100 and one true inclusion had an *LR* less than 100 (limited support). One false exclusion (not shown) was obtained at a DNA amount of 200 due to an elevated stutter allele.

In contrast to the single source samples, the DNA amount of low-level contributors (less than 200 RFU) determined by STRmix<sup>™</sup> during deconvolution is generally lower than the average peak height. This can be expected because STRmix<sup>™</sup> is able to account for stochastic variation and alleles which have dropped below the laboratory's analytical threshold when calculating DNA amount. APH is calculated using only the alleles which have been detected above the laboratory's analytical threshold. Additional information from the other contributor can also help provide data about the entire profile. This creates a trend of lower DNA amounts than average peak heights for low-level contributors in mixtures instead of the variability of DNA amount compared to average peak height in single source samples.

![](_page_6_Figure_4.jpeg)

Figure III-B3: Zoomed-in plot of Log(LR) versus number of unique minor alleles for 2-person mixtures

For the 2-person mixtures, false inclusions were obtained when the lower level contributor displayed nine or less unique minor alleles (see Figure III-B3). Two of the false inclusions had six unique minor alleles and *LR*s greater than 100. One true inclusion, with 6 unique minor alleles, had an *LR* less than 100 (limited support). One false exclusion (not shown) was observed for a sample with 26 unique minor alleles due to an elevated stutter allele.

![](_page_7_Picture_0.jpeg)

![](_page_7_Figure_2.jpeg)

![](_page_7_Figure_3.jpeg)

For the 2-person mixtures, false inclusions were obtained when STRmix<sup>M</sup> determined the lower-level contributor to be 2% (See Figure III-B4). Two of the false inclusions had *LR*s greater than 100. One true inclusion at 2% had an *LR* less than 100 (limited support). One false exclusion (not shown) was observed for a sample with an 8% lower-level contributor due to an elevated stutter allele.

# C. 3-person mixture samples

The plots below (Figures III-C1 to III-C3 and Figure III-C5) are "zooms" of the lower-level contributors from the 3-person mixture samples to provide a more in depth evaluation of the area where false inclusions/exclusions intersect. As expected, and demonstrated in the previous validation, higher-level contributors produce fully concordant inclusions/exclusions. It is important to note that each plot also demonstrates a significant number of full exclusions (Log(LR) < -5 and Log(LR) = 0) at low levels that are not shown. Full versions of each plot are available in the electronic data.

![](_page_8_Picture_0.jpeg)

![](_page_8_Figure_2.jpeg)

![](_page_8_Figure_3.jpeg)

False inclusions were obtained for low-level contributors with average peak heights of 176 RFU or less, however, all *LR*s were below 100 (see Figure III-C1). One true inclusion at an average peak height of 133 RFU had an *LR* less than 100 (limited support). Two false exclusions, one with *LR* = 0 due to an elevated stutter allele (not shown) and one qualitative exclusion, were obtained with average peak heights of 152 and 142 RFU.

![](_page_8_Figure_5.jpeg)

![](_page_8_Figure_6.jpeg)

False inclusions were obtained for low-level contributors with DNA amounts of 193 or less, however, all *LR*s were below 100 (see Figure III-C2). One true inclusion at a DNA amount of 125 had an *LR* less than 100 (limited support). Two false exclusions, one with LR = 0 due to an elevated stutter allele (not shown)

![](_page_9_Picture_0.jpeg)

and one qualitative exclusion, were obtained with DNA amounts of 168 and 150. Similar to the 2-person mixture samples, the DNA amount determined by STRmix<sup>™</sup> during deconvolution is generally lower than average peak height for low-level contributors (less than 200 RFU). This may be due to the low number of unique minor alleles detected above threshold for some of the 3-person mixture samples.

![](_page_9_Figure_3.jpeg)

Figure III-C3: Zoomed-in plot of Log(LR) versus number of unique minor alleles for 3-person mixtures

False inclusions were obtained for low-level contributors with 10 unique minor alleles or less, however, all *LR*s were below 100 (see Figure III-C3). One true inclusion with 10 unique minor alleles had an *LR* less than 100 (limited support). Two false exclusions were obtained, one with 11 unique minor alleles but an LR = 0 due to an elevated stutter allele (not shown) and one qualitative exclusion with two unique minor alleles.

![](_page_9_Figure_6.jpeg)

Figure III-C4: Full plot of Log(LR) versus number of unique minor alleles for 3-person mixtures

![](_page_10_Picture_0.jpeg)

While the "zoom" of the unique minor alleles plot for the three person mixtures (Figure III-C3) shows the level where false inclusions/exclusions were observed, it did not provide a complete picture of the reliability of this parameter for indicating the suitability of a contributor for comparison. The full plot (Figure III-C4) demonstrates that the true inclusions/exclusions did not present the expected pattern from this type of data analysis. The *LR* from the known contributors did not climb to a steady plateau at a high *LR* and the *LR* from the non-contributors did not fall to a steady plateau of full exclusion (*LR* = 0 represented graphically as *LR* = -30). This is most likely due to the lack of unique minor alleles in higher order mixtures due to masking by other contributors or locations in stutter positions. Therefore, higher-order mixtures (3-person or more) may be better assessed using average peak height or DNA amount.

![](_page_10_Figure_3.jpeg)

Figure III-C5: Zoomed-in plot of log (LR) versus percent minor contributor for 3-person mixtures

False inclusions were obtained for low-level contributors at 16% or less, however, all *LR*s were below 100 (see Figure III-C5). One true inclusion at 13% had an *LR* less than 100 (limited support). Two false exclusions, one with LR = 0 due to an elevated stutter allele (not shown) and one qualitative exclusion, were obtained at 14% and 9%.

![](_page_11_Picture_0.jpeg)

![](_page_11_Figure_2.jpeg)

**Figure III-C6**: Full plot of log(*LR*) versus percent minor contributor for 3-person mixtures

While the "zoom" of the percent minor contributor plot for the 3-person mixtures (Figure III-C5) shows the level where false inclusions/exclusions were observed, it did not provide a complete picture of the reliability of this parameter for indicating the suitability of a contributor for comparison. The full plot (Figure III-C6) demonstrates that the true inclusions/exclusions did not present the expected pattern from this type of data analysis. The *LR* from the known contributors did not climb to a steady plateau at a high *LR* and the *LR* from the non-contributors did not fall to a steady plateau of full exclusion (*LR* = 0 represented graphically as *LR* = -30). This is most likely due to the lack of effect on the percentage of a contributor when altering the total average peak height and/or input DNA. For example, a 10% contributor in a low-template mixture (e.g., 100 pg) will typically produce a much lower *LR* than a 10% contributor in a high-template mixture (e.g., 750 pg) due to stochastic variation and drop out. Therefore, higher-level mixtures (3-person or more) may be better assessed using average peak height or DNA amount.

# D. 4-person mixture samples

The plots below (Figures III-D1 and III-D2) are "zooms" of the contributors from the 4-person mixture samples to provide a more in-depth evaluation of the area where false inclusions/exclusions intersect. As expected, and demonstrated in the previous validation, higher level contributors produce fully concordant inclusions/exclusions. It is important to note that each plot also demonstrates a significant number of full exclusions (Log(LR) < -5 and Log(LR) = 0) at low levels that are not shown. All data and/or full versions of each plot are available in the electronic data. As with the 3-person mixture samples, the percent contributor and number of unique minor alleles plots for the 4-person mixture samples did not demonstrate the expected pattern for this type of data analysis indicating they may not be reliable parameters for determining the suitability of a contributor for comparison. These plots will not be included or further discussed in this section.

![](_page_12_Picture_0.jpeg)

![](_page_12_Figure_2.jpeg)

Figure III-D1: Zoomed-in plot of log(LR) versus APH (in RFU) for 4-person mixtures

False inclusions were obtained for contributors with average peak heights of 254 RFU or less, and false inclusions with *LR*s greater than 100 were obtained at 163 RFU or less (see Figure III-D1). Three true inclusions at average peak heights of 130 RFU, 185 RFU, and 243 RFU had *LR*s less than 100 (limited support). One false exclusion (not shown) was obtained with an average peak height of 249 RFU due to an elevated stutter peak modeled as a true allele.

![](_page_12_Figure_5.jpeg)

Figure III-D2: Zoomed-in plot of log(LR) versus DNA amount for 4-person mixtures

False inclusions were obtained for contributors with DNA amounts of 200 or less, and false inclusions with *LR*s greater than 100 were obtained at 81 or less (see Figure III-D2). Three true inclusions at DNA amounts of 25, 75, and 93 had *LR*s less than 100 (limited support). One false exclusion (not shown) was obtained with a DNA amount of 181 due to an elevated stutter peak modeled as a true allele.

![](_page_13_Picture_0.jpeg)

#### E. 5-person mixture samples

The plots below (Figures III-E1 and III-E2) are "zooms" of the contributors from the 5-person mixture samples to provide a more in-depth evaluation of the area where false inclusions/exclusions intersect. As expected, and demonstrated in the previous validation, higher-level contributors produce fully concordant inclusions/exclusions. It is important to note that each plot also demonstrates a significant number of full exclusions (Log(LR) < -6 and Log(LR) = 0) at low levels that are not shown. All data and/or full versions of each plot are available in the electronic data. As with the 3-person and 4-person mixture samples, the percent contributor and number of unique minor alleles plots for the five person mixture samples did not demonstrate the expected pattern for this type of data analysis indicating they may not be reliable parameters for determining the suitability of a contributor for comparison. These plots will not be included or further discussed in this section.

![](_page_13_Figure_4.jpeg)

Figure III-E1: Zoomed-in plot of log(LR) versus average peak height (in RFU) for 5-person mixtures

False inclusions with *LR*s greater than 100 were obtained for contributors with average peak heights of 397 RFU or less (see Figure III-E1). Three true inclusions at average peak heights of 358 RFU, 220 RFU, and 138 RFU had *LR*s less than 100 (limited support). No false exclusions were observed.

![](_page_14_Picture_0.jpeg)

![](_page_14_Figure_2.jpeg)

Figure III-E2: Zoomed-in plot of log(LR) versus DNA amount for 5-person mixtures

False inclusions were obtained for contributors with DNA amounts of 362 or less, and false inclusions with *LR*s greater than 100 were obtained for contributors with DNA amounts of 275 or less (Figure III-E2). Three true inclusions at DNA amounts of 156, 25, and one had *LR*s less than 100 (limited support). No false exclusions were observed.

![](_page_15_Picture_0.jpeg)

#### IV. Conclusions

The data obtained in this study demonstrated that each indicator (average peak height, DNA amount, percent contributor, and number of unique minor alleles) can provide information to help an analyst determine the suitability of a DNA profile and/or its specific contributors. However, some indicators are more informative than others, depending on the number of contributors. Single source samples are best evaluated using the total number of autosomal loci with results and average peak heights. The lower-level contributors in 2-person mixtures are best evaluated by the number of unique minor autosomal alleles present and their average peak heights. With higher-order mixtures (3-, 4-, and 5-person), percent contributor and the number of unique minor alleles are less informative and it is more difficult to calculate average peak height for specific contributors due to the sharing and/or dropout of alleles. For these samples, the DNA amount determined by STRmix<sup>™</sup> during deconvolution can be used to help an analyst decide the suitability of a particular contributor. While there was some variability observed between average peak height and DNA amount for low-level contributors, overall, these values appear to coincide (see Figure IV-1).

![](_page_15_Figure_4.jpeg)

**Figure IV-1:** DNA amount versus APH (in RFU) of all true contributors (single source through 5-person mixtures)

![](_page_16_Picture_0.jpeg)

To apply the conclusions reached by this study, it is recommended to incorporate the information below in the laboratory's standard operating procedure as guidelines and/or limitations.

- Single source samples with results at less than 5 autosomal loci and average peak heights of less than 125 RFU are not expected to provide an *LR* above 100.
- 2-person mixtures where the lower-level contributor displays less than 10 unique minor autosomal alleles and average peak heights of less than 125 RFU are not expected to provide an *LR* above 100.
- For 3- and 4-person mixtures, contributors with DNA amounts less than 200 may produce false inclusions. For 5-person mixtures, contributors with DNA amounts less than 375 may produce false inclusions.
- These recommendations have been made using results from single amplification data. Replicate amplifications are expected to reduce the levels at which false inclusions have been observed.

![](_page_17_Picture_0.jpeg)

#### V. Appendix A – Test Drive

To verify the effectiveness of the results and recommendations made in this study, a set of 9 samples of various targets and number of contributors was interpreted by two analysts. Each analyst interpreted the samples prior to reading the report and recommended SOP edits and then interpreted the samples again after reading the report and recommended SOP edits. The results of these evaluations are below.

#### Sample A

Truth: 2-person mixture, 300 pg target, 1:15 ratio

Interpretation before: Both analysts agreed that the DNA profile was a 2-person mixture and the minor contributor was uninterpretable.

Interpretation after: Both analysts agreed that the DNA profile was a 2-person mixture and the minor contributor was uninterpretable.

Evaluation: While there was no disagreement or change in the analysts' interpretations of this sample, the data reported in this study and the recommended SOP edits provided improved guidance and support for the decision to report the minor contributor as uninterpretable.

#### Sample B

Truth: 2-person mixture, 600 pg target, 1:20 ratio

Interpretation before: Analyst 1 determined that the DNA profile was from either 2- or 3contributors, while Analyst 2 determined that the DNA profile was from 3 contributors. However, both analysts agreed that only the upper-level contributor was interpretable.

Interpretation after: Both analysts agreed that the DNA profile was from 2 contributors and the upper-level contributor was interpretable. However, Analyst 1 concluded that the lower-level contributor was uninterpretable while Analyst 2 concluded that the lower-level contributor was interpretable.

Evaluation: Based on the data, the conclusion determined by Analyst 2 after reading the study and recommended SOP edits was the most accurate. In truth, the sample is a 2-person mixture and the *LR*s for both contributors are  $10^{35}$  and  $10^{20}$ . Although the minor contributor DNA profile had more than 10 unique minor alleles with an APH greater than 125 RFU, the data did show inconsistency in the peak heights of the minor contributor with multiple minor alleles in stutter positions. In this instance, it was understandable for an analyst to exercise caution in interpretation of the lower-level contributor. If the sample had been interpreted by STRmix<sup>TM</sup> as a 3-person mixture, little change was observed in the *LR* for the two true contributors and the maximum false inclusion was obtained at an *LR* showing limited support.

#### Sample C

Truth: 3-person mixture, 500 pg target, 20:10:1 ratio

Interpretation before: Both analysts agreed that the DNA profile was from 3 contributors and the 2 upper-level contributors were interpretable.

Interpretation after: Both analysts agreed that the DNA profile was from 3 contributors and the 2 upper-level contributors were interpretable.

![](_page_18_Picture_0.jpeg)

Evaluation: While there was no disagreement or change in the analysts' interpretations of this sample, the data reported in this study and the recommended SOP edits provided improved guidance and support for the decision to report the 2 upper-level contributors as interpretable.

#### Sample D

Truth: 4-person mixture, 100 pg target, 1:2:3:4 ratio

Interpretation before: Analyst 1 concluded that the entire profile was uninterpretable. Analyst 2 concluded that the DNA profile was from either 3 or 4 contributors and only the upper-level contributor was interpretable. The additional 2 or 3 contributors were uninterpretable.

Interpretation after: Both analysts agreed that the entire profile was uninterpretable.

Evaluation: The data obtained in this study successfully informed Analyst 1 and Analyst 2 that the overall peak heights for this sample were near or below the recommended level for interpretation (DNA Amounts: 218, 150, 100, 50) and may result in a false inclusion. While 3 of the true contributors produced *LR*s above 100, a false inclusion for the mixture was also obtained at an *LR* of 846.

# Sample E

Truth: 4-person mixture, 100 pg target, 3:3:2:1 ratio

Interpretation before: Analyst 1 concluded that the entire profile was uninterpretable. Analyst 2 concluded that the DNA profile was from either 3 or 4 contributors and only the upper-level contributor was interpretable. The additional 2 or 3 contributors were uninterpretable.

Interpretation after: Both analysts agreed that the entire profile was uninterpretable.

Evaluation: A similar result as Sample D was obtained for this sample, however, the *LR* of the highest false inclusion for this mixture was less than 100.

# Sample F

Truth: 4-person mixture, 400 pg target, 1:3:5:10 ratio

Interpretation before: Analyst 1 concluded that the DNA profile was from 3 contributors and only the upper-level contributor was interpretable. Analyst 2 concluded that the DNA profile was from 4 contributors and the 2 upper-level contributors were interpretable.

Interpretation after: Both analysts agreed that there were 4 contributors and the upper-level contributor was interpretable. However, Analyst 1 also concluded that the second upper-level contributor was interpretable and Analyst 2 did not agree. Both analysts agreed that the 2 lower-level contributors were uninterpretable.

Evaluation: Whether interpreted as a 3-person or 4-person mixture, no false inclusions were obtained from this sample. Additionally, minimal differences were obtained for the *LR* of the 3 upper-level contributors and all *LR*s were well above 100 despite the lowest DNA amounts of 268. There was some disagreement between the two analysts on the second upper-level contributor. Although the DNA amounts for the second and third contributors were greater than 200, close evaluation of the profile indicates that the second and third contributor's alleles were below the laboratory's recommended stochastic range and not easily distinguished from each other. At this level, it can be expected to see some variability between analysts as it may be dependent upon the amount of suspected allele sharing between contributors.

![](_page_19_Picture_0.jpeg)

# Sample G

Truth: 5-person mixture, 1000 pg target, 10:10:5:1:1 ratio

Interpretation before: Both analysts agreed that the DNA profile was from either 4 or 5 contributors. Analyst 1 concluded that the 2 upper-level contributors were interpretable. Analyst 2 concluded that the 3 upper-level contributors were interpretable.

Interpretation after: Analyst 1 concluded that the DNA profile was from 4 contributors and Analyst 2 concluded that the DNA profile was from either 4 or 5 contributors. Both analysts agreed that the 2 upper-level contributors were interpretable.

Evaluation: While there was some disagreement regarding the number of contributors, the *LRs* for the 3 upper level contributors are nearly the same for either scenario. However, when interpreted as a 5-person mixture a false inclusion with an *LR* of 428 was obtained. Both analysts came to similar conclusions after reading this study and the recommended SOP edits indicating that the information not only provided guidance regarding the contributor's DNA amounts (1056, 781, 250, 50, 25) but also added consistency between individuals.

#### Sample H

Truth: 5-person mixture, 300 pg target, 20:1:1:1:1 ratio

Interpretation before: Both analysts concluded that the DNA profile was from 3 contributors and only the upper-level contributor was interpretable.

Interpretation after: Both analysts concluded that the DNA profile was from 3 contributors and only the upper-level contributor was interpretable.

Evaluation: The target for this sample was a 5-person mixture, however, not all contributors were represented in the results. The highest number of alleles observed at two loci was five. Additionally, no effect was observed in the *LR* for the upper-level contributor. False exclusions at *LR*s of 562 and 1,210 were obtained when the sample was interpreted as a 5-person and 3-person mixture, respectively. While the results of this study and recommended SOP edits did not change the conclusions of either analyst, they did provide additional guidance and support. The 4 lower-level contributors resulted in DNA amounts of less than 100 when run as a 5-person mixture, and the 2 lower-level contributors resulted in DNA amounts of less than 200 when run as a 3-person mixture.

# Sample I

Truth: 5-person mixture, 300 pg target, 1:1:2:2:2 ratio

Interpretation before: Analyst 1 concluded that the entire mixture was uninterpretable. Analyst 2 concluded that the DNA profile was from 5 contributors and the 4 upper-level contributors were interpretable.

Interpretation after: Both analysts agreed that the entire mixture is uninterpretable.

Evaluation: While no false inclusions were obtained for this sample, the data and SOP edits successfully informed Analyst 1 and Analyst 2 that some of the contributors were near or below the recommended level for a 5-person mixture (DNA Amounts: 1150, 587, 400, 343, 84). Additionally, both analysts determined that more than 5 contributors may have been present and interpretation of the mixture was not possible.

![](_page_20_Picture_0.jpeg)

Overall, this "test drive" of the study results and recommended SOP edits demonstrated that analysts are correctly assessing the different levels at which a profile and/or specific contributors become complex. While not all samples resulted in identical conclusions from both analysts, the variability occurred at expected levels and without consequence to accuracy. Most importantly, the consistency between the reported conclusions from Analyst 1 and Analyst 2 increased from only 3 identical conclusions to 6 identical conclusions.

#### VI. Appendix B – Citations

DC DFS, Forensic Biology Unit (2016). Internal Validation of the Applied Biosystems<sup>™</sup> Globalfiler<sup>™</sup> PCR Amplification Kit using the Applied Biosystems<sup>™</sup> 3500/3500xL Genetic Analyzer.

DC DFS, Forensic Biology Unit (2017). Part II: Internal Validation of STRmix Version 2.4.

DC DFS, Forensic Biology Unit Standard Operating Procedure (FBS31) – GlobalFiler™ Interpretation.

DC DFS, Forensic Biology Unit Standard Operating Procedure (FBS32) – GlobalFiler™ Data Analysis Using STRmix™.

DC DFS, Department Operation Manual (DOM04) – Validating Technical Procedures.

Federal Bureau of Investigation (2020). Quality Assurance Standards for Forensic DNA Testing Laboratories.

Scientific Working Group on DNA Analysis Methods (SWGDAM) (2017). SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories.

Scientific Working Group on DNA Analysis Methods (SWGDAM) (2015). SWGDAM Guidelines for the Validation of Probabilistic Genotyping Systems.

Scientific Working Group on DNA Analysis Methods (SWGDAM) (2016). SWGDAM Validation Guidelines for Forensic DNA Analysis Methods.

Scientific Working Group on DNA Analysis Methods (SWGDAM) (2018). Recommendations of the SWGDAM Ad Hoc Working Group on Genotyping Results Reported as Likelihood Ratios.

![](_page_21_Picture_0.jpeg)

# Updates as of January 14, 2022 (original version approved on October 5, 2020):

The Zoom Study: Additional Guidelines for Interpretation of Mixtures and Low Level Data Using GlobalFiler<sup>™</sup> on the 3500/3500xL and/or STRmix<sup>™</sup> 2.4 validation report was updated in January 2022 to provide clarity to the content of the report.

A summary of the updates is listed below:

Page 2: Defined terminology for true inclusion/exclusion and false inclusion/exclusion.

Page 5: A title was added to Figure III-A5.

Page 16: The title for Figure IV-1 was updated.

**Page 21**: URLs for the FBI Quality Assurance Standards and SWGDAM documents in Appendix B were removed because those are not permanent URLs.

# Throughout the document:

Figures were enlarged for easier viewing.

Figures which did not previously have captions were labeled with captions for clarity.

Grammatical and non-substantive fixes were made.

Updates to this validation report did not result in any change to standard operating procedures. All DC DFS FBU standard operating procedures for the use and interpretation of results from the STRmix<sup>™</sup> software and GlobalFiler<sup>™</sup> kit remain the same after updates to this document.

Updates to this validation report were reviewed and approved by:

Clark Jaw, FBU Technical Leader (Primary)

Date