Questions Received during Workshop

- Guideline 3.6.2.1
- Does "suitable for comparison and statistical analysis" mean "above the stochastic threshold"?

- In the analysis process, we determine the loci that are suitable for statistical analysis before comparisons are made
- But:
- Should the comparison be done between the reference and the mixture at those loci that were previously decided to be not useful for statistical analysis?

 Stochastic values rely on validations in which a sister allele drops out. How do you establish drop-out values for something like Y-STRs which have a single peak at a locus?

- Using peak height ratios alone, one can deduce likely major/minor contributors at a locus.
- If that is the case, why is it also necessary to establish defined major/minor ratios, such as 3to-1 or 4-to-1, etc.?

- What is the weight/significance of a MUST in a guideline?
- Still not "auditable", right?

- There is a critical need to inform analysts that the terminology "not retroactive" <u>does not</u> mean that forensic scientists are permitted or should let previous conclusions stand that would change (either outcome or frequency) under a new mixture interpretation policy.
- Fairness within the criminal justice system should require that a new report be issued. Shouldn't this be one of the "musts"?
- Shouldn't forensic scientists be required to inform both prosecutors and defense attorneys of the "new results"?

- Selecting a confidence level (or determining the amount of useable data) is really a subjective opinion. Setting the level too high is ignoring a great deal of valid data (98-99%)
- What level is appropriate??
- We are seeing arguments among analysts about the appropriate confidence levels. What guidance can you provide?
- Should a lab report out a conclusion and state the confidence level in the conclusion?

 Given that low DNA/RFU levels increase the stochastic effects and the challenges associated with mixtures, has any consideration been given to making a guideline/recommendation on the quantity of input DNA (a minimum limit)?

• Is there commercially available software that consultants use for forensic calculations?

• What should lab do about older cases that are coming up to trial now?

• What about parent/child mutations to major/minor components?

- When calculating mixture ratio for 2-person mixtures (based on the 4-allele loci) should that calculation be considered accurate if only one 4allele locus is in the mixture?
- In other words, should I say in my SOP that I can only accurately calculate a mixture ratio if I can base it on X number of loci?

 If alleles are present in an electropherogram that are below the stochastic threshold, should I determine the number of contributors or the mixture ratio?

 Are there any statistics on PHRs for single individuals that could help in interpreting contributors from multiple individuals in a mixture?

- In the example give by Mike Adamowicz, major/minor ratios were: 5.32, 3.75, and 4.2
- Can anything be done using the variance of these major/minor ratios around the average for a mixture?

 How do you handle assumptions for the number of contributors and mixture ratios, when crime scene involves parents and one or more biological children?

- Deducing a second contributor, 2 alleles observed
- Known is homozygote: obligate of 2nd
- Known is heterozygote: "cannot deduce"
- "Cannot deduce" not always true (barring any abnormalities, i.e. primer site mutations, degradation, etc.)
- No question asked

Question 18 – for Joanne Sgueglia

- Is it appropriate to set an analytical threshold based on peak shape/morphology of true alleles?
- Low-level DNA with poor amplification morphology at 50 RFU, so you set analytical threshold at 75 RFU to avoid them
- Stochastic threshold:
 - Setting a stochastic threshold based on a mixture study – your thoughts?

- Is it appropriate to use a 3-person mixture to determine the stochastic threshold?
- Are there advantages or disadvantages?

• Do you know if/when SWGDAM Guidelines will be incorporated into QAS document?

 Can you elaborate on Guideline 3.5.2.1 (Re: assigning alleles to major contributor when no assumptions to number of contributors are made)?

- Question for the group:
- Are labs applying stutter percentages to peaks when doing mixture analysis?
- If so, is the value the same as that used for filtering stutter with single-source samples?

• Is Todd's mixture program available?

- Low-level sample with no more than 2 alleles above the analytical threshold and/or stochastic threshold with other possible peaks below analytical threshold.
- Would you report this as a mixture?

- When will the next edition of John Butler's book be available?
- Advanced Topics in Forensic DNA Typing: Methodology was completed last week and will be available as part of the Promega meeting in October 2011

 If your lab has set the analytical threshold, should you "look" below for mixture interpretation?

• Is there any guidance on a minimum number of loci to draw an inclusion/exclusion statement?

 On average, how much extra time should a lab expect to spend handling a mixture (compared to a non-mixture)?

 How often (what percentage of samples handled by a lab) does a lab deal with "mixtures" whether it is related to sexual assault samples or touch DNA samples?

- Can you safely assume you have no drop-out at a locus that has 2 alleles present below the stochastic threshold when the whole profile indicates a single contributor?
- Similarly, can you make the same assumption at loci with 4 alleles under the stochastic threshold in a 2-person mixture?

• What is the ideal peak height ratio for 3 or more contributors in a mixture?

- If you have the possibility of allelic dropout in an indistinguishable mixture, can you ever exclude anyone?
- In other words, if you can't use a locus to include, should you not use it to exclude either?

 Can any interpretation be made for mixtures of more than 2 people where you cannot confidently assume the number of contributors, which contains multiple peaks above the stochastic threshold and multiple peaks between the analytical threshold and stochastic threshold across multiple loci?

- Are there any recommendations regarding setting guidelines for when a complex mixture should not be interpreted or compared based on potential number of contributors?
- In other words, possibly 3 or more contributors or 4 or more contributors
- Is there a sense of the direction of the forensic community [in this effort to draw a line in the sand and not interpret data past it]?

- Our former Technical Leader advocated the use of a "load" as one criteria to determine whether a locus could be used for statistical analysis. The load was defined as the sum of the peak heights at a locus.
- ...
- Do you think such an approach has merit?

- In our lab, our analytical threshold is 100 RFUs. We also have a minimum analysis threshold (50 RFU) and label peaks with an asterisk and can use them for exclusionary purposes.
- For calculating CPI, we only use loci with all peaks above the analytical threshold (100 RFU).
 For CPE, we use loci with peaks above the minimum analysis threshold (50 RFU, not able to use for CPI). Is having two different thresholds for CPI and CPE statistically valid?

- What is your opinion about employing CPE as your mixture statistic? Do you think it appears to a jury as more weighted than the CPI value?
- Example is Michael's presentation: 1 in 1,670 Caucasians included (CPI) versus 99.940% Caucasians excluded

- If [you] find suspect on multiple pieces of evidence, in order to say he is on each piece do you need to put a stat for each piece?
- What about if he is found on multiple stains from the same piece of evidence? (20 stains from sheet and he is a contributor to all 20) Do I need to do a stat for all 20 stains?