Draft Minutes
Scientific Advisory Committee
Subcommittee on CE Protocols
August 10, 2009
Department of Forensic Science, Classroom 1

Subcommittee Members Present

Dr. Norah Rudin, chair
Dr. Dan Krane
Dr. John Butler

Virginia Department of Forensic Science ("DFS") Staff Present

Mr. Brad Jenkins (Program Manager, Forensic Biology)
Ms. Elizabeth Ballard (Forensic Scientist, Senior)
Dr. Susan Greenspoon (Forensic Molecular Biologist)
Dr. Katie Hall (Forensic Laboratory Specialist VI)
Dr. Dave Barron (Director of Technical Services)

Individuals Present at Some Point During the Meeting
Dr. Dale Carpenter (Scientific Advisory Committee)
Mr. Peter Marone (DFS Director)

Call to Order

Dr. Norah Rudin, subcommittee chair, called the meeting to order at approximately 9:35am.

New Business

The subcommittee adopted the agenda for the meeting.

Issues that qualify as a "clear public danger" or "specific accreditation violation"

Discussion ensued that these might be better interpreted as critical issues, as "clear public danger" was difficult to discern. Dr. Krane indicated three areas for discussion that may fall under this heading: limit of detection ("LOD"), mixture interpretation, and stutter. Dr. Butler had none. Dr. Rudin discussed the need to have enough negative control or reagent blank. Each issue was discussed in detail.

LOD

Dr. Krane expressed a desire that LOD be determined on a run-by-run basis. Dr. Butler questioned the feasibility of doing so. Mr. Jenkins made it clear that the DFS approach to LOD followed from the method approved by the Scientific Advisory Committee ("SAC") Y-STR subcommittee for use with

electropherogram data. Discussion ensued regarding the most appropriate estimation of LOD based on the maximum observed noise (2x maximum noise vs. 3x maximum noise). Discussion ensued regarding how often the LOD will be reevaluated by DFS. Dr. Butler indicated that annual review is plenty, but certainly when the laser is replaced.

Mixture Assessment/Major-Minor

Heterozygous Peak Balance validation summary: Committee members agreed that data for sample 11 at CSF1PO should not be utilized for statistical analyses, as it exhibits a known triallelic pattern at this locus. Mr. Jenkins agreed to do so. Questions were raised regarding Sample 1 at vWA and the use of the modified internal lane standard. All committee members agreed that data from the statewide performance checks should be mined to demonstrate the effect of the new ILS on the peak height ratios reported in the validation summary. Dr. Krane suggested that, rather than the minimum observed, the average, standard deviation, and 95% confidence interval should be reported in the protocol (to replace current Table 3 under 8.2.4. of the draft protocol). Dr. Rudin questioned if scatterplots of any lower amounts of DNA input should be included in the summary.

Major/Minor Assessment: Dr. Greenspoon discussed approaches used by other laboratories. Dr. Butler indicated that the DFS result follows the German Stain Commission report. Dr. Krane expressed concern regarding applying statistics to a minor profile (see 8.2.7.7.4 of the draft manual). Mr. Jenkins assured the subcommittee that this would be a rare occurrence and the determination would be done in the "blind" portion of the analysis. The committee was satisfied with the response by Mr. Jenkins. Dr. Krane would like to see such clarification in the text at that location in the manual.

Re: Further discussion ensued regarding the 33.5% cutoff for determining major/minor contributors. Discussion was largely tabled until after lunch, when the data could be examined. Dr. Krane made several suggestions to clarify the text of the Major/Minor Assessment validation summary regarding samples. Dr. Rudin suggested that, in the future, DFS may wish to consider further analysis using samples with less 1 ng total DNA.

The committee took a mid-morning break.
Meeting resumed at approximately 10:37am by Dr. Rudin.

Further discussion ensued regarding the major/minor determination and DNA mixtures. No resolution to the cutoff percentage was made at this time, but was deferred until afternoon when a review of the data could be completed.

Subcommittee members discussed 8.2.7.7.7 of the draft version of the manual in regards to mixtures. Drs. Krane and Rudin asked questions in regards to the wording in this section including: allelic dropout, rare alleles, and majority. Mr.

Jenkins clarified the intent of the protocol. Dr. Rudin suggested DFS may wish to consider revising this wording. Mr. Jenkins agreed that he would clarify the wording of the paragraph. Dr. Rudin expressed concern regarding the use of "at least four callable loci". Dr. Greenspoon indicated that a study was completed at the request of a previous committee which resulted in this policy. Dr. Krane suggested revising the cutoff based on the statistics, not based on the number of loci. Drs. Krane and Rudin suggest a review of data in regards to this issue. Dr. Rudin also expressed a problem with reporting inconclusive results *with reference to* reference profiles and suggested alternative wording. Dr. Greenspoon clarified the current report wording, which Drs. Rudin and Butler believe is good.

Discussion ensued regarding the laboratory's training of analysts for this transition to CE. Dr. Rudin asked Dr. Carpenter to add the training update to tomorrow's Scientific Advisory Committee meeting agenda.

Other issues

Samples

Dr. Rudin was concerned about the location of samples on the injection plate. Ms. Ballard clarified the plate loading format for automated sample processing, regarding maintaining the plate format from extraction through injection on the CE. Dr. Rudin was satisfied with that approach, but requested clarification in the protocol, especially in regard to manual setups.

Number of contributors

Dr. Krane discussed section 8.2.3 of the draft manual in regards to the ability to determine the number of contributors in a mixture. Mr. Jenkins agreed to add clarification to the manual at this location that indicates peak heights and number of alleles are taken into account when determining the number of contributors to a mixture.

Section 8.2.2 of the draft manual was raised by Dr. Krane but discussion was tabled until later.

Workflow

Dr. Rudin requested clarification regarding documentation in the casefile regarding blindedness of analysis. Mr. Jenkins and Ms. Ballard clarified. Dr. Rudin indicated that DFS may consider time-stamping notes.

Stutter

Dr. Krane suggested that section 8.2.7.4.2 (last sentence) be clarified. He indicated the lack of correspondence between Table 4 of the protocol and Table 1 of the Stutter validation summary. Dr. Krane suggested that conventional rounding be utilized.

The committee took a break for lunch at approximately 11:35am.
Meeting resumed at approximately 12:20pm by Dr. Rudin.

Dr. Rudin asked the committee members for any issues that must be changed before implementation. Dr. Krane suggested that Table 4 (8.2.7.4.2 of draft protocol) must be concordant with the validation summary.

Discussion turned to more minor issues.

Suggestions for "Optimization"

Dr. Krane gave comments on the validation summaries:

Concordance
It was suggested that the DFS data using non-expired reagents be used. DFS will do so.

Sensitivity
Discussion ensued regarding the reporting of a range for optimal signal. There was disagreement among subcommittee members.

Precision
Dr. Krane asked if DFS was intending to redo the precision study when new versions of GeneMapper ID ("GMID") software are adopted. Mr. Jenkins indicated that this was a requirement of ISO accreditation. Dr. Krane suggested listing the version number of GMID here and elsewhere. Minor additional edits to the summary were discussed.

Major/Minor Assessment
Discussion ensued regarding distinguishing a minor contributor from stutter. Dr. Butler referred to the International Society for Forensic Genetics recommendation #6[1] where peaks in the stutter position must be included in the CPE/CPI calculation if the stutter peaks and minor alleles are of similar height. Drs. Butler and Rudin agreed that this was not a "show-stopper". Wording changes were suggested in this regard.

Heterozygote Peak Height Ratio
Specific data were requested for review at the next break (Samples 1, 5, and 11) regarding potential binding site mutations. It was agreed that Sample 11 would be discarded at CSF1PO (or an asterisk added) explaining the known tri-allelic pattern at that locus. Additional minor revisions were requested.

Statewide Performance Check
The GeneMapper ID version (3.2.1) should be indicated. Discussion ensued regarding optimal signal reported as 0.31 ng – 1.25 ng template DNA. Dr. Krane would prefer 0.62 ng. DFS should ensure that all instances of this optimal range are consistent (protocols, validation summaries, etc.)

Environmental Samples

[1] *Forensic Science International* 160(2006): 90-101.

Dr. Krane requested wording changes and clarification in a few instances. Discussion ensued regarding the allelic dropout observed in all three room temperature samples. Dr. Greenspoon indicated that the samples are going to be recreated for future use due to the low amount of remaining material. Dr. Krane indicated he has devised a means of calculating the slope of the "ski slope" if DFS wishes to use it.

Non-Probative Casework
It was indicated that the lowest input amount demonstrated here was 0.65ng total DNA. If DFS wishes to utilize an optimal range of 0.31 – 1.25ng, additional non-probative samples would need to be run.

Limit of Detection (cont.)
Dr. Krane again indicated his preference would be for run-specific LODs. Mr. Jenkins indicated he did not forsee DFS doing so, but revisiting the LODs on a yearly basis, perhaps. Dr. Krane then suggested DFS consider allowing calculation of the LOD on a run-by-run basis if indicated.

Stutter
Drs. Rudin and Krane agree that conventional rounding should be utilized. Edits were suggested to Tables 1 and 2. It was indicated that the protocol should be edited to reflect the need to evaluate n-(2 repeat) and n+(1 repeat) stutter. Dr. Greenspoon indicated that an additional table would be added to the protocol.

Mixtures
Typographical errors were indicated and removal of the phrase "and the origin of the cutoff value" suggested.

The subcommittee took a break to review data.
Meeting resumed by Dr. Rudin.

Limit of Detection (cont.)
Drs. Rudin and Krane agreed that the blue and green data appears appropriate. The 247 bp peak that defined the maximal noise in the yellow channel will be revisited by DFS, as it is now known to be an artifactual peak . It was understood that minor changes to the data may occur.

Peak Height Ratio (cont.)
Dr. Rudin indicated that sample 5 at FGA could be a binding site mutation. DFS could choose to omit this from the statistics as an outlier or keep it as a representation of the population. Dr. Krane indicated that the lower bound was not established by these data points. Dr. Krane indicated that Sample 1 at vWA looked okay.

Optimal Injection Parameters
Dr. Rudin indicated that the LOD must be revisited if longer injection times or increased amplification product are used. Dr. Rudin suggested run-specific LODs or run-specific LODs for these (non-default) injections. Dr. Butler suggested that noise is expected to

stay the same for increased injection times, but the stochastic threshold would be expected to increase. Dr. Butler suggested analysis of the data. If it is determined that the noise didn't change, then no more study would be needed in this regard.

Suggestions for "Optimization" of Protocols

Amplification
Clarification and rewording was suggested in several locations. Ensure consistency in target DNA range with validation summaries.

Capillary Electrophoresis
The version of GMID used should be indicated. Sections 2.7.4, 2.7.4.4, and 2.8.2.3.7 of the draft manual were discussed earlier today. Citations for several figures were suggested by Dr. Butler.

Analysis of Capillary Electrophoretic Data
DFS was asked to provide the Appendix referred to in 7.3.7.3.1 of the draft manual. Mr. Jenkins did so. DFS was asked to clarify what would necessitate adjusting the red LOD referred to in 7.3.7.3.1 of the draft manual. DFS was asked to clarify section 7.3.9.4.1.3 of the draft manual. Ms. Ballard did so. Dr. Krane suggested alternative wording to consider. Dr. Krane requested a definition of "called alleles" in 7.3.9.4.4 of the draft manual. Dr. Rudin suggested DFS think about whether anything needs to be done with peaks in the negative control below the threshold. Dr. Krane requested clarification in the protocol regarding remaking the positive control. It was clarified that the intent of the wording was to allow for repreparing the injection cocktail. Dr. Rudin requested clarification in the manual regarding printing of evidentiary *and reference* samples for the case file.

Interpretation of PowerPlex® 16 PCR Amplification Results
Wording changes were suggested and typographical errors noted. Dr. Butler indicated that more specific chromosome location data are available in a March 2006 *Journal of Forensic Science* article. Regarding section 8.2.4, Dr. Rudin suggested a list of factors taken into account when determining the number of contributors to a mixture be delineated in the protocol here. Mr. Jenkins indicated he would take that suggestion into consideration. Dr. Krane asked that Table 3 be replaced by Table 3 from the Heterozygous Peak Balance validation summary. Mr. Jenkins indicated that he will give that consideration. Dr. Rudin requested clarification of "careful consideration" in section 8.2.7.4 of the draft manual. Mr. Jenkins listed common factors to take into account. Dr. Rudin suggested defining these in the protocol. Dr. Krane suggested that section 4.2.5.4 of the Databank draft manual is better and the two should be consistent. Similarly, Dr. Rudin suggested listing factors to evaluate in the protocol to aid in distinguishing between stutter and a minor allele. It was suggested to update Table 4 for scientific rounding, ensuring consistency between the protocol and the validation summary. As indicated previously, the up-stutter table was also suggested to be added here. Refer to previous discussion regarding several sections in this chapter of the protocol. Dr. Rudin suggested less vague wording for 8.2.12 regarding the reagent blank.

The subcommittee took a mid-afternoon break.
Meeting resumed by Dr. Rudin at approximately 3:45pm.

Interpretation of PowerPlex® 16 PCR Amplification Results (cont.)
Discussion ensued regarding the reporting inconclusive results at a single locus. Mr. Jenkins clarified current protocol. Varying opinions were discussed regarding the two-tiered approach by DFS. Dr. Rudin suggested another approach. Mr. Jenkins agreed to think about the other approaches discussed.

Discussion ensued regarding the need for a QA person to review the protocol. Dr. Krane indicated that an external review could be sought, as there is not yet a replacement for Ms. Deborah Friedman. Dr. Carpenter suggested the QA persons in his laboratory system have DNA backgrounds and may be available.

Dr. Carpenter requested that Director Marone join the meeting to clarify DFS position regarding provisional vs. non-provisional approval.

Discussion returned to Section 8 of the draft manual (Interpretation of the PowerPlex 16 PCR Amplification Results). Section 8.2.14 was discussed. Clarification was requested regarding section 8.3, in particular the next to last sentence. It was suggested that Figure 6 be utilized to describe the intent. In the Figure 6 example, it was suggested that the phrase "may not be solely from the victim" be used (see D16S539 and D8S1179) so as not to suggest unintended bias.

Director Marone arrived.

Dr. Carpenter requested clarification of the position of DFS regarding provisional and actual approval. Director Marone indicated that approval or disapproval should be rendered, as provisional approval would be analogous to disapproval in terms of DFS going online with this technology. Dr. Rudin indicated that she forsees no problem with databank approval. Other options were explored such as phone-in meetings, etc. Dr. Butler indicated that no "deal breakers" were found by the committee. Dr. Rudin suggested compiling a list of 3-5 major issues and obtaining DFS comment on those issues. Dr. Krane suggested that numerous small issues were discussed which, in total, could be construed as "deal breakers". Dr. Carpenter reminded the subcommittee that this negotiation has been done in good faith. Approval could be given with the understanding that the SAC would be shown documentation regarding the changes made.

The main issues were compiled by the subcommittee to include:

1. LOD. Use maximum noise x 2 instead of maximum noise x 3 to estimate LOD. Mr. Jenkins agreed to do so.
2. Heterozygous Peak Balance – discussion ensued regarding 33.5% vs. 33% vs. "approximately". Mr. Jenkins indicated that DFS can use 33%.
3. Four callable loci – Dr. Rudin was happy with DFS thinking about the issue. Mr. Jenkins noted that DFS would review the data and reiterated that any change

would go back on a previous committee recommendation. Dr. Krane suggested that the current wording could be approved tomorrow, but that the issue should be revisited in January with a presentation by DFS.

4. Stochastic Threshold – Dr. Butler indicated that the SWGDAM recommendations should come out in January and are expected to include advocating a stochastic threshold. The committee suggested DFS reconsider this point in six months.
5. Interpretation of Reagent Blanks – Dr. Rudin was okay with putting this issue on the agenda for discussion in January. Mr. Jenkins suggested DFS adopt the more stringent guideline of reamplifying/reextracting as needed. Further thought will be required in situations where the reagent blank or sample is limited. Dr. Rudin suggested revisiting the topic in January if a better option is found.

All concur that the protocol should be recommended for approval with these main issues as suggested improvements. Dr. Rudin suggested seeking a QA person's comments by the January meeting as well.

Dr. Rudin will prepare a draft report for the Scientific Advisory Committee tomorrow. Dr. Krane would like it to be known that a very careful evaluation has been completed.

Public Comment

No public comment.

The meeting was officially adjourned at approximately 4:45pm.