B12 Database Samples Warranting a Closer Look and Examination of the D8S1179 Locus

Amanda J. Hoffman, MS, Marshall University Forensic Science Center, 1401 Forensic Science Drive, Huntington, WV 25701; and Jason Chute, MSFS, 1401 Forensic Science Drive, Huntington, WV 25701*

After attending this presentation, attendees will have closely observed two samples that originally produced profiles requiring additional examination for questionable allele calls at the D8S1179 locus. Attendees will see that in addition to standard practices employed at the Marshall University Forensic Science Center (MUFSC), steps were needed to determine the true genotypes for each of these samples in question.

This presentation will impact the forensic science community by enlightening attendees that analysts should be very aware that profiles produced may require additional examination due to inconsistencies or questionable calls. It is imperative to ensure that the true DNA profiles for each sample will be uploaded into local, state, and federal databases.

The original samples were previously spotted on Whatman® FTA® blood cards at the West Virginia State Police Forensic Laboratory (WVSPFL) and processed using the direct amplification method with PowerPlex® 16 HS employed at the MUFSC. These two database samples required more lab work due to problems with an allele call, a peak height ratio, and dropout at the D8S1179 locus.

Sample A and Sample B (generic sample names used in place of identifying the barcodes used in processing) were extracted and amplified using PowerPlex® 16 HS, PowerPlex® 16, and AmpFlSTR® Identifiler® Plus. Sample A was initially direct amplified using the Powerplex® 16 HS kit resulting in an Off Ladder (OL) peak at the D8S1179 locus with a height of 704 RFU, a size of 225.08, and a calculated call of a 12.3. When comparing the sample with the ladder, the math came out to: 225.79-225.08=0.71 on one side of the ladder and 225.08-221.77=3.31 on the other side of the ladder, resulting in the call of a 12.3. This microvariant has been seen at the MUFSC six times and on STR base three times, leading an analyst to believe this could be a true call. The calculations resulting in the 12.3 call did seem a little far off from the normal OL/microvariant calculation normally seen. Normally, when calculated, the OL/microvariants are closer to a "1" nucleotide difference; however, based on the analysis, the sample appeared to have an allele call of 8,12.3 at D8S1179. Similar results were obtained from a cutting that was extracted on the EZ1® robot resulting in the 8 and 12.3 at D8S1179.

Sample A was subsequently amplified with Identifiler® Plus with a 1.0µl load of the neat extract, with a target of 1.933ng. With this kit, the allele in question fell into the 13 bin, resulting in a 8,13 call for the D8S1179 locus. In this testing, the 13 allele had a height of 1688 RFU and a size of 143.84. These Identifiler® Plus kit results led to the previous Powerplex® 16 HS kit results being questioned. Additional processing of Sample A was completed by the assistance of the National Institute of Standards and Technology (NIST) to obtain a profile that could be submitted to the West Virginia State Police for upload into the Combined DNA Index System (CODIS). The final and true allele calls for Sample A at the D8S1179 locus were determined to be an 8 and 13.

Sample B originally appeared to be homozygous at the D8S1179 locus with a 12,12 allele call, but close examination of the data caused the analyst to question whether there was possible allelic dropout. This sample would normally pass because the homozygous allele passed the stochastic threshold at the MUFSC. More laboratory work concluded that this sample did in fact have a sister allele of a 14 at this locus. This sample had to be extracted and amplified using PowerPlex® 16 HS, PowerPlex® 16, and AmpFlSTR® *Identifiler*® Plus to obtain the true genotype.

This study will present the steps and processes taken to obtain successful and true profiles and the initial reasons for further examination of each sample. Each profile produced will be highlighted and explained for each amplification of the two samples. It is recommended that persons in the field are familiar with DNA analysis of single-source reference samples, direct amplification, and traditional methods of extraction and amplification, as well as peak height ratios and dropout.

DNA, Database, Microvariant