A52 Validation and Comparison of the AmpFℓSTR® Identifiler® Plus PCR Amplification Kit to Identifiler®, MiniFiler™, and Yfiler® for the Pinellas County, Florida Forensic Laboratory

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After attending this presentation, attendees will gain an understanding of the Identifiler® Plus amplification chemistry's performance in various studies as compared to the Identifiler®, MiniFiler™, and Yfiler® chemistries.

This presentation will impact the forensic science community by providing information about capabilities and limitations of Identifiler® Plus under various conditions and on different instruments. By comparing this amplification chemistry to other available chemistries, forensic laboratories will have advanced insight into which chemistry may be best suited for their specific needs.

Using these protocols, amplification of DNA samples must occur prior to capillary electrophoresis in order to generate the desired human identification profile required for presentation in court proceedings. The type of amplification chemistry used in this process can affect the profile obtained from the sample. The Identifiler® Plus amplification chemistry from Applied Biosystems was selected for this study as it contains a reportedly improved master mix, with AmpliTaq Gold® DNA Polymerase already added to the mix as well as an enhanced formula to reduce Polymerase Chain Reaction (PCR) inhibition of samples. The kit is designed to provide improved sensitivity to enable use with low template DNA samples, while also allowing for more effective analysis of mixture samples. Identifiler® Plus uses fluorescent multi-color dyes to analyze 15 loci with alleles with overlapping size ranges. It follows the same design as Identifiler®, but is an improvement on the MiniFiler™ chemistry, which only amplifies nine loci. Yfiler amplifies 17 Y-STR loci, so direct comparisons could only be made for certain studies.

An internal validation of Identifiler® Plus was performed for the Pinellas County Forensic Laboratory in Largo, Florida. Eight validation studies were performed, including accuracy, precision, recovery, linearity/sensitivity, range, mixture, carryover, and ruggedness. Accuracy was determined from non-probative samples that simulated casework, mixture samples created from positive controls, and all positive controls used in the validation. Precision was measured from the standard deviations of 48 allelic ladders and the 250 base pair (bp) peak of all samples in the validation. A sensitivity study was used to measure linearity, in that a range of DNA concentrations were amplified in order to establish the optimal concentration for successful amplification. 9947A and 007 control samples were used to create mixture ratios ranging from 19:1 to 1:19. Ruggedness was determined by amplifying the samples using three different thermal cyclers. All negative controls used in the validation were analyzed for contamination. The results were compared to the results of samples amplified with Identifiler® as well as results from previous Identifiler®, MiniFiler™, and Yfiler® validations. All samples were amplified for 28 and 29 cycles to determine the ideal PCR cycle number for Identifiler® Plus. Additionally, the limit of detection and sensitivity samples were run on three different genetic analyzers (two 3130 genetic analyzers and one 3130xl genetic analyzer) to ascertain any discrepancies between the instruments.

The results of the validation supported the use of 28 PCR cycles with Identifiler® Plus. The samples displayed greater sensitivity than Identifiler® and were comparative to MiniFiler™ and Yfiler®. Results of the precision study fell within manufacturer recommendations. Samples demonstrated 100% accuracy and no contamination was present in any of the negative controls used in the validation. Samples run at different times and on different thermal cyclers were consistent with expected results and with each other. Mixture samples could be resolved and full profiles of the minor contributor were generated down to 6:1 and 1:9. The samples run on the 3130xl genetic analyzer displayed greater sensitivity than samples run on the 3130 genetic analyzer, but limit of detection stayed the same for both instruments.

Identifiler® Plus, Validation, Amplification

 ** Presenting Author*