Validation of AmpF*l*STR™ Profiler Plus™ And Cofiler™ Profiling Systems for Use in Forensic Casework

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Forensic laboratories have used Polymerase Chain Reaction (PCR) extensively for the past 5-10 years. New technology has lead to the production of new kits that utilize PCR to examine Short Tandem Repeat (STR) loci within sample DNA. This differs somewhat from the earlier PCR-based testing in that the DNA analysis is based on length polymorphisms rather than sequence polymorphisms. Also, 13 loci plus amelogenin are typed thus leading to a highly discriminatory test.

Currently, we are validating 2 manufactured kits in the laboratory in accordance with TWGDAM guidelines for use in forensic casework. These 2 kits were chosen as they include all 13 of the CODIS loci. Initial studies were performed using AmpF*l*STR™ Profiler Plus™ and Green I™ until Cofiler™ became available. These kits are being validated on the ABI 310 Genetic Analyzer. In order to evaluate consistency and reproducibility of the kits and the instrument, different specimens from the same individual (hair, blood, buccal swabs, semen/vaginal swabs) were analyzed. A total of 10 individuals were tested, 5 male and 5 female. The analysis of DNA obtained from the different biological sources yielded consistent, reliable results. Each specimen type was also extracted using either an organic procedure or the Chelex method and these results were compared as well. For sensitivity studies, dilutions ranging from 2 ng to 0.1 ng of known type DNA were examined. DNA from different biological sources was also examined. Mixture studies were performed using known concentrations of DNA in ratio of 16:1 to 1:16 to determine the lower level of detection for the minor component in a mixture. For these studies, mixtures of DNA from individuals whose profiles shared numerous alleles as well as mixtures of DNA from individuals whose profiles were quite dissimilar were used. In general, the minor component could be determined out to an approximately 1:8 ratio. Studies were also performed to determine the amplification capabilities of DNA (in a biological sample) that was deposited on a variety of substrates where the DNA was of a known type. The samples were extracted organically, with Chelex, or both. As expected, DNA deposited on most sources was typeable. In general, the Chelex reaction yielded better results than the organic extraction. The precision of the instrument was determined by calculating the mean base pair size ± S.D. for each allele in 52 ladders. A comparison was made using the calculated mean for each allele from the analyzed data to the published mean. This study was performed using 2 different lots of columns and polymer. Our "in laboratory" percent stutter was also determined. This was done utilizing known single source samples that were run with several different lot numbers of polymer and columns. These results were also similar to the published data. We are in the process of completing our validation study and are currently performing heterozygous peak height ratio studies, environmental studies, family studies, and nonhuman studies. These will be completed in accordance with TWGDAM guidelines. However, the results of our validation thus far indicate the STR profiling is reliable, sensitive, and specific and that this system may be utilized for forensic casework.