A50 Internal Validation of AmpfℓSTR® Minifiler™ Amplification Kit for Metropolitan Police Department

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After attending this presentation, attendees will understand the requirements and principles behind an internal validation for a start-up forensic laboratory.

This presentation will affect the forensic community by providing DNA requirements and recommendations relative to the validation for Metropolitan Police Department of the AmpfℓSTR® Minifiler™ amplification kit that other forensic laboratories may utilize in their own validation efforts.

The AmpfℓSTR® Minifiler™ kit was designed to reliably type degraded, inhibited, low-copy number nuclear DNA samples. Samples with low copy number or inhibition may now generate full or partial profiles which were previously unable to be genotyped. This amplification kit is able to perform this function by generating smaller polymerase chain reaction products for eight loci. These loci include D7S820, D13S317, D16S539, D21S11, D2S1338, D18S51, CSF1PO, FGA and Amelogenin. Internal validation is a required process for every forensic laboratory before a new kit or instrument can be used for casework. The goal of this project was to validate the Minifiler™ amplification kit for Metropolitan Police Department for use in DNA identification while also utilizing VALID™ software. The VALID™ software provides detailed steps and suggestions to take for completion of this project. The tests performed included a precision, sensitivity, accuracy and reproducibility and a mixture study. Samples originally were analyzed at 40 rfu and results from the individual tests required that this number be increased. Throughout the course of the validation, while using pristine DNA samples, there were a significant number of artifacts seen. These included significant off-ladder alleles, -A and secondary stutter which is recognized by Applied Biosystems at the D13 locus. After the completion of the tests required by VALID™ a study was performed to try and type samples that showed poor genotyping results using the Identifiler™ amplification kit. The results from this study, eight samples with target concentrations of 0.025 and 0.05 ng/ul, were 83 new alleles that were previously not typed using Identifiler™. This validation demonstrated excellent concordance with the previous alleles typed using Identifiler™ and demonstrates that the amount of DNA required for reliable typing may be a range between 0.025 ng/ul and 0.05 ng/ul with an RFU threshold of 85 to 100 rfu.

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