



A59 Internal Validation of the AmpF Φ STR[®] Identifiler[®] Plus PCR Amplification Kit and Comparison to Identifiler[®] for the Boston Police Department Crime Laboratory

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After attending this presentation, attendees will be well-informed of the performance of the Identifiler[®] Plus amplification chemistry on reference and mock casework samples as compared to the Identifiler[®] amplification chemistry.

This presentation will impact the forensic community by comparing and contrasting the performance of Identifiler[®] versus Identifiler[®] Plus, and discussing the improvements and limitations of Identifiler[®] Plus so DNA laboratories may determine which amplification chemistry is best suited for their individual needs.

Amplification is an important step in the process of generating a DNA profile, allowing as little as a few cells to generate millions to billions of copies so that the sample DNA can be detected. The amplification kit selected can affect the quantity and quality of results obtained. Therefore, great care should be taken in choosing the DNA amplification kit that is best suited for the needs of the laboratory. The Identifiler[®] Plus amplification chemistry is an improvement over the first generation Identifiler[®] amplification kit. While both kits amplify the same loci with the same primer sequences in the same concentrations, the Identifiler[®] Plus amplification kit has demonstrated improved performance on severely inhibited samples, greater sensitivity, improved performance on mixture samples, a cleaner baseline, and a reduced number of artifacts due to an improved primer manufacturing process. The Identifiler[®] Plus amplification kit is able to amplify a lower target amount with the same or better sensitivity as the Identifiler[®] amplification kit. The Identifiler[®] Plus amplification time is also one hour shorter than Identifiler[®] time.

An internal validation was performed for the Identifiler[®] Plus amplification kit at the Boston Police Department Crime Laboratory. The results of the validation support a protocol of amplification for 28 PCR cycles with a DNA target input of 0.75ng and an injection time of 10 seconds on a 3130xl genetic analyzer. The validation studies confirmed the precision stated by the manufacturer for the kit and showed full concordance in allele calls with the Identifiler[®] amplification kit. The sensitivity studies showed that full singlesource profiles above the analytical threshold could be obtained from a target input of 0.125ng, but not all peaks were above the stochastic threshold at that target. Reproducibility in allele calls and peak heights was also seen in samples that were amplified two weeks prior. Mixture studies demonstrated that the Identifiler[®] Plus amplification kit was able to detect a mixture sample when the minor component was present as 5% of the total DNA and that a full profile from the minor component was often generated when present as 25% of the mixture sample. A full profile for the minor component was rarely obtained when the minor component was present as 10% of the total mixture. No contamination was found in the negative controls throughout the validation studies.

The Identifiler[®] Plus internal validation instituted a double threshold for analysis, with an analytical and stochastic threshold put in place. This varied greatly from the single calling threshold that was in place with the Identifiler[®] amplification kit. Amplification with Identifiler[®] Plus on touch DNA and degraded samples provided more allele calls than when the samples were amplified with Identifiler[®], due to both the increased sensitivity and performance of the kit, as well as the lower analytical threshold in place for calling. However, many alleles that had previously been able to be used for statistical purposes in the Identifiler[®] amplification samples were now falling between the analytical and stochastic threshold for the Identifiler[®] Plus samples. These alleles were no longer able to be used in statistics and could be used for exclusions only. Nevertheless, the Identifiler[®] Plus amplification kit provided more information overall and should be considered for use over the Identifiler[®] amplification kit.

Identifiler[®] Plus, Validation, Comparison