



A49 Validation of the Applied Biosystems® 3500 Genetic Analyzer With a Comparison of the Identifiler® Plus and PowerPlex® 16 HS Amplification Kits

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The goal of this presentation is to demonstrate the ability of the Applied Biosystems 3500 genetic analyzer to produce complete and accurate forensic DNA profiles with a variety of sample types under a variety of different conditions. It will also provide a comparison of two commonly used amplification kits from the two major manufacturers of forensic analytical supplies for consideration by forensic laboratories. A denaturation study shows the ability for laboratories to validate procedures without the denaturation and snap cooling step prior to a run on an instrument such as the AB 3500. This has the potential to increase efficiency in forensic casework analysis. This validation can also serve as a starting point for future validations of the AB 3500 genetic analyzers.

This presentation will impact the forensic science community by validating the Applied Biosystems 3500 genetic analyzer and the comparison of the PowerPlex® 16 HS and Identifiler® Plus amplification kits on this instrument will provide the forensic science community with information that will allow laboratories to make informed decisions about the kits and equipment that would be best suited to their needs.

Applied Biosystems® 3500 series of genetic analyzers is the newest technology available for forensic casework analysis and has not yet been widely adopted by the forensic community. As such, there are limited studies available that involve the performance of amplification kits commonly used on these instruments. The Anne Arundel County Crime Lab is currently upgrading from an AB 310 genetic analyzer to an AB 3500 genetic analyzer. The internal validation of this AB 3500 included a comparison of the PowerPlex® 16 HS and Identifiler® Plus amplification kits using casework samples and the manufacturer's recommended protocols to determine if one had any advantages over the other, when used in conjunction with the AB 3500. Analytical and stochastic thresholds were calculated for both a 7-second and a 15-second injection time, which were validated for casework analysis on this AB 3500. Precision, contamination, sensitivity, concordance, reproducibility, and stutter studies were also performed during this validation. The AB 3500 generated complete and accurate forensic DNA profiles with both kits and both injection times over a wide range of DNA target amounts at amplification. However, the AB 3500 was able to generate more complete profiles for lower level single source samples and mixture samples using PowerPlex® 16 HS, when the same target amount of DNA was used at amplification in each kit. Powerplex® 16 HS was also shown to generate higher peaks across each dye channel on the AB 3500 when compared to Identifiler® Plus. Aside from this difference in sensitivity, there were no significant differences in performance between the PowerPlex® 16 HS and Identifiler® Plus amplification kits on the AB 3500. A denaturation and snap cooling study showed that the denaturation and snap cooling step in the laboratory's standard operating procedures had no effect on the data produced by the AB 3500 with either kit. As a result of this validation, the Anne Arundel County Crime Lab will continue to use the PowerPlex® 16 HS amplification kit for casework analysis on their newly validated AB 3500 with a standard operating procedure that does not include the denaturation and snap cooling step prior to an instrument run. Extended use of the POP 4 polymer pouch was also investigated beyond the recommended 7 days on the instrument after opening. Storing the POP 4 polymer in the refrigerator after opening, when it is not in use on the instrument, was shown to extend the useful life of the polymer by several weeks. Future studies may include manipulation of the amplification cycles with Identifiler® Plus to see how the sensitivity of this kit can be improved for lower level single source samples and mixture samples. There is also a tendency for the D13 and D5 loci to dropout more frequently in lower level samples than other loci with both PowerPlex® 16 HS and Identifiler® Plus on both the AB 3500 and the AB 310, which was used in this validation for concordance studies. Future studies could be undertaken to determine the cause of this imbalance.

AB 3500, PowerPlex® 16 HS, Identifiler® Plus