A51 Validation of the Applied Biosystems® 3500xL With PowerPlex® 16 HS

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After attending this presentation, attendees will understand the procedures of the internal validation performed at the Philadelphia Police Forensic Science Bureau on the Applied Biosystems® 3500xL with PowerPlex® 16 HS. Attendees will learn which settings were selected to optimize the use of this chemistry with the instrument as well as the results of the various studies performed.

This presentation will impact the forensic science community by the sharing of information in regards to the validation of a relatively new platform to the field of forensics, the Applied Biosystems® 3500xL Genetic Analyzer. This instrument will soon replace the Applied Biosystems® 3130 Genetic Analyzer as the primary capillary electrophoresis instrument used in forensic DNA laboratories. This validation also incorporated a frequently used amplification chemistry, Promega Corporation's PowerPlex® 16 HS.

An important aspect of forensic science is the need to validate new chemistries and instrumentation for use with STR analysis. An internal validation must be performed on these new methods and technologies prior to implementation in laboratory standard operating procedures. This validation must demonstrate the ability of the procedure to obtain reliable results, the ideal conditions to obtain these results, and the limitations for this new procedure. An internal validation was performed for the Philadelphia Police Forensic Science Bureau through the National Institute of Justice Technical Assistance Program on the use of PowerPlex® 16 HS amplification chemistry with the Applied Biosystems® 3500xL Genetic Analyzer. The 3500 Genetic Analyzer is a relatively new platform to the field of forensic science and offers many advantages over the previously used Applied Biosystems® 3130, such as, an improved mechanical pump, new laser technology, more consistent temperature control, prepackaged consumables, and reduced power requirements. Data files are also saved in .HID format and analyzed with GeneMapper® ID-X (Applied Biosystems). The PowerPlex® 16 HS amplification chemistry, released in 2009, added hot-start Taq directly to their mastermix, eliminating the need to purchase this reagent separately, an advantage over the previously used PowerPlex® 16 kit. PowerPlex® 16 HS also offers the ability to perform direct amplification procedures, as well as an increased ability to perform in the presence of inhibitors.

This internal validation incorporated a variety of studies to demonstrate the reliability and reproducibility of this instrument with the PowerPlex® 16 HS chemistry. These studies included the determination of an appropriate analytical threshold through SWGDAM approved methods, a stochastic threshold, an injection time and voltage that provided optimal peak heights and peak height ratios, a sensitivity range to provide the optimal input amount of target DNA, heterozygosity ratios, and stutter percentages for comparison to manufacturer-recommended values. Studies were run simultaneously on both the 3130xL and 3500xL instruments for the duration of the validation study. The results from the 3500xL were compared with data collected from the 3130xL to demonstrate concordance. Results collected for various other studies were also compared between the two platforms including stutter ratios and mixture interpretations. Samples were also analyzed to demonstrate reproducibility across multiple amplifications and runs, and were compared with the expected results from previously analyzed non-probative casework samples. These non-probative casework samples demonstrated various extraction methods and substrates typically encountered in the Philadelphia Police Forensic Science Bureau. The comparison of these results demonstrates instrument concordance.

Overall, results from the 3500xL were observed to contain peaks of a greater intensity when compared to the 3130xL. The instrument also provided accurate profiles with few artifacts across a wide range of input target DNA. Based on the findings of these studies, specific settings were recommended to be incorporated into the standard operating procedure of the Philadelphia Police Forensic Science Bureau. These settings included a set analytical threshold across all dye channels, a stochastic threshold value to assist in the determination of true homozygote peaks, an optimal target DNA range, laboratory specific stutter ratios, and mixture interpretation guidelines. The instrument produced reliable results with the PowerPlex® 16 HS amplification chemistry and the use of this chemistry with the 3500xL was recommended for future use.

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