Developmental Validation of the PowerPlex® 16 HS System

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The PowerPlex® 16 HS System is an updated version of the PowerPlex® 16 System; while the primers and dyes remain unchanged, it introduces an enhanced buffer system that includes hot-start *Taq* DNA polymerase and ensures robust performance. Due to the modification of the reaction mix, a multi-laboratory developmental validation study was completed to document performance capabilities and limitations for the revised assay. Data within this validation was generated by eight laboratories and served as the basis for the following conclusions: Genotyping of single-source samples was consistent across a large range of template DNA concentrations with most laboratories obtaining complete profiles at 62.5pg. Mixture analyses showed that over 90% of minor alleles were detected at 1:9 ratios. Optimum amplification cycle number was ultimately dependent on the sensitivity of the detection instrument and could be adjusted to accommodate a range of DNA template concentrations. Reaction conditions including volume and annealing temperature as well as the concentrations of primers, *Taq* DNA polymerase, and magnesium were shown to be optimal and able to withstand moderate variations without affecting multiplexed STR amplification. The robustness of the enhanced buffer systems tolerates a much higher concentration of common PCR inhibitors and allows for direct amplification from DNA collection card punches. Finally, data from non-probative samples and concordance studies showed consistent results when comparing the PowerPlex® 16 HS System with the PowerPlex® 16 System as well as other commercially available systems. The combined sensitivity and flexibility of the PowerPlex® 16 HS System make it a great option for both databasing and casework labs.