B120 A Validation Study of Applied Biosystems™ VeriFiler™ Plus Polymerase Chain Reaction (PCR) Amplification Kit

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Learning Overview: The goal of this presentation is to introduce attendees to data from the internal validation of a new megaplex PCR amplification kit and the issues encountered prior to the kit's submission to the National DNA Index System (NDIS) for approval.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing another megaplex PCR amplification kit that includes internal quality control markers that monitor PCR efficiency, sample quality, and the possible presence of inhibitors on a per-sample basis.

Several megaplex PCR amplification kits exist that include the expanded Combined DNA Index System (CODIS) Core Loci required by the Federal Bureau of Investigation (FBI) as of January 1, 2017. However, only six PCR kits contain internal quality control markers, and only one kit was approved by NDIS before this validation was completed. The Applied Biosystems™ (AB) VeriFiler™ Plus (VFP) PCR Amplification Kit is a new 6-dye Short-Tandem Repeat (STR) multiplex assay for the amplification of human genomic DNA.[1] The VFP PCR kit amplifies 23 autosomal STR loci (including the expanded CODIS Core Loci), two internal quality control markers (IQCS and IQCL), one insertion/deletion marker on the Y chromosome (Y indel), and amelogenin (sex determining marker). The IQCS and IQCL are two synthetic targets, one low molecular weight and one high molecular weight, which are amplified simultaneously with the sample. The internal quality control system evaluates the success of the PCR and indicates sample quality.

The studies performed at the North Louisiana Criminalistics Laboratory (NLCL) were in accordance with the FBI's Quality Assurance Standards for an internal validation. The studies included sensitivity, precision, accuracy, contamination, concordance, known and non-probative samples, and mixture samples. Extracted DNA was quantified using the Quantifiler® Trio DNA Quantification Kit. All samples were run on the Applied Biosystems™ 7500 Real-Time PCR System and analyzed using Human Identification (HID) Real-Time Software v1.3. Samples were amplified on GeneAmp™ PCR System 9700 thermal cyclers using two-stage cycling parameters with max ramping for a total of 29 cycles. Amplified product was run on the Applied Biosystem™ 3500 Genetic Analyzer using 3500 Series Data Collection Software 4 (DC v4). GeneMapper™ ID-X Software v1.6 was used for data analysis.

During the validation of the VFP PCR kit, several reproducible and non-specific artifacts were characterized that could not be attributed to the kit or the 3500 Genetic Analyzer. The cause of these artifacts was determined to be the DC v4. Specifically, the Signal Optimization (SO) feature is intended to reduce the peak height variation caused by the instrument optics and injection conditions primarily for 24-capillary instruments. Since the VFP validation was performed on 8-capillary 3500 instruments, it was necessary to complete a performance check with the SO feature disabled. The performance check demonstrated that contamination, sensitivity, precision, and accuracy were not significantly affected with the SO feature disabled. However, the non-specific artifacts observed in the validation data were greatly reduced, which correlated with a cleaner baseline.

The studies in the validation demonstrate the VFP PCR kit is a robust, reliable, and sensitive STR multiplex assay for the amplification of specific loci in human genomic DNA. Sensitivity and linearity are maintained when samples are of ample quantity and are consistent with the developmental validation. Single-source samples show reproducibility of genotype, and the mixture samples cover numerous contributor ratios and sample conditions. Concordance was demonstrated between the current PCR kit and the VFP PCR kit. Precise and accurate results were obtained using the VFP PCR kit with a lack of contamination. Although a number of artifacts were observed in the validation study, the performance check verified the artifacts were attributed to the DC v4 Software and not the VFP PCR kit.

Reference(s):

1. ThermoFisher Scientific. VeriFiler™ Plus PCR Amplification Kit User Guide, Revision B.0.

DNA Analysis, STRs, Validation

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