



Pathology Biology Section – 2009

G26 Internal Validation of Quantifiler™ DUO DNA Quantification Kit and AmpFℓSTR® Yfiler™ PCR Amplification Kit

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After attending this presentation, attendees will learn about the methods and results from the validation study of two Y specific kits performed at the Institute of Forensic Science of Puerto Rico.

This presentation will impact the forensic community by communicating the methods and results from a validation study of two available forensic kits. Y-locus specific kits are an important forensic tool to aid in the discrimination of the male contribution in a sample, such as rape cases.

The DNA-Serology Laboratory of the Institute of Forensic Sciences of Puerto Rico is the only Latin American laboratory accredited by ASCLD-LAB. Y-STR's have become an important forensic tool in cases in which male-male or male-female mixtures arise, such as rape cases. Y-locus STR's have also gained importance in the clarification of erroneous Amelogenin tests from autosomal STR amplification kits. Quantitation of human male DNA and Y-STR analysis are not currently performed in-house. Therefore, this study was designed to validate two commercially available forensic kits, Quantifiler® Duo DNA Quantification, and AmpFℓSTR® Yfiler™ PCR Amplification for in-house quantitation of human male DNA and Y-STR detection. These kits were validated for use with one real-time PCR instrument model for DNA quantitation (ABI Prism® 7500 Sequence Detection System) and two capillary electrophoresis instrument models (ABI Prism® 3130xl and 3100-Avant Genetic Analyzers) for Y-STR detection.

Quantifiler® Duo DNA Quantification Kit is designed to simultaneously quantify the total amount of amplifiable human DNA and human male DNA in one reaction. The quantification assay combines three 5' nuclease assays, namely: target-specific human DNA assay, target-specific human male DNA assay, plus an internal PCR control (IPC). The human target is Ribonuclease P RNA Component H1 (RPPH1) located at 14q11.2 and 140 bases long is detected by TaqMan® MGB probe labeled with VIC dye. The male target is the sex-determining region Y (SRY) located at Yp11.3 and 130 bases long is detected by TaqMan® MGB probe labeled with FAM dye. The internal PCR control is a synthetic sequence not found in nature. It is 130 bases long and is detected by TaqMan® MGB probe labeled with NED dye. AmpFℓSTR® Yfiler™ PCR amplification kit is a short tandem repeat multiplex for human male-specific DNA amplification that includes the European minimal haplotype loci (DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392, and DYS393), the SWGDAM recommended loci (DYS438 and DYS439), and additional highly polymorphic loci (DYS437, DYS448, DYS456, DYS458, DYS635, and Y GATA H4) for a total of 17 Y-STR loci in a single PCR reaction.

Internal validation studies included: precision, accuracy, sensitivity, male:male mixture evaluation, female:male mixture evaluation, stutter determination for each locus, as well as forensic casework. Forensic casework includes a double-rape case, an incest case, a proficiency test from Collaborative Testing Services, Inc., and a case with null Amelogenin typed with AmpFℓSTR® Identifier™ PCR Amplification kit. In addition to these studies, 106 samples were used to create a Y-STR database for Puerto Rico. All validation study data is being analyzed and will be discussed.

Internal validation of available DNA typing kits for human identification allows for the evaluation of a procedure's efficiency, reliability, performance characteristics, and limitations. The internal validation of the Y-locus specific kits (AmpFℓSTR® Yfiler™ and Quantifiler® Duo) will allow the Puerto Rico Institute of Forensic Science DNA laboratory to save time and money, as well as the ability to offer reliable male DNA typing services.

Y-STR, Male Quantitation, Yfiler™