PROMEGA POWERPLEX® Y23 INTERNAL VALIDATION AND ITS APPLICATION TO KINSHIP STUDIES IN LABORATORIO DE GENÉTICA MOLECULAR- CRUZ VITAL

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Peak heights (RFU), reproducibility, internal standards, instrument precision and possible contamination on the results of the amplification of samples processed with routine methods were evaluated in the laboratory. Materials and Methods: capillary blood samples on FTA cards (GE Healthcare), DNA extraction from FTA cards as recommended by the manufacturer, amplification with Promega PowerPlex® Y23 in two Verity thermal cyclers, Capillary Electrophoresis performed on the 3130 and 3500 Genetic Analyzers from Applied Biosystems. Two different samples from male individuals, which were tested in another similar laboratory for comparison, were employed. These samples were processed by 3 different operators, three times for each of the two thermal cyclers. Negative controls were also processed with cuts of a clean FTA card. 2800 M cell line was amplified as positive control. Sample electrophoretic set up included the ILS-500 Y23 and Y-23 ladder. Data Collection V 2.0 and GeneMapper V 3.2 (Applied Biosystems) software were used to collect and edit data. A throughout comparison was made considering every result from the 3 different operators. The peak heights were evaluated and its average was 7164 RFU. Reproducibility was calculated by comparing the profiles obtained by analysts with those obtained in another laboratory and resulted in 100% agreement; the average allele size in base pairs was calculated to demonstrate precision: the standard deviation calculated was lower than 0.5 base pairs. No contamination was observed in negative controls.

Results from this validation study using PowerPlex® Y23 from Promega and the fact of having full concordance with GHEP-ISFG and GCIH intercomparison exercises proof that the methodology used in the Laboratorio de Genética Molecular de Cruz Vital is reproducible, accurate and reliable.

References:
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