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Internal validation of two new retrotransposons-based kits (InnoQuant® HY and InnoTyper® 21) at a forensic lab

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ABSTRACT

Obtaining a genetic profile from pieces of evidence collected at a crime scene is the primary objective of forensic laboratories. New procedures, methods, kits, software or equipment must be carefully evaluated and validated before its implementation. The constant development of new methodologies for DNA testing leads to a steady process of validation, which consists of demonstrating that the technology is robust, reproducible, and reliable throughout a defined range of conditions. The present work aims to internally validate two new retrotransposon-based kits (InnoQuant® HY and InnoTyper® 21), under the working conditions of the Laboratório de Polícia Científica da Polícia Judiciária (LPC-PJ).

For the internal validation of InnoQuant® HY and InnoTyper® 21 sensitivity, repeatability, reproducibility, and mixture tests and a concordance study between these new kits and those currently in use at LPC-PJ (Quantifiler® Duo and GlobalFiler™) were performed.

The results obtained for sensitivity, repeatability, and reproducibility tests demonstrated that both InnoQuant® HY and InnoTyper® 21 are robust, reproducible, and reliable. The results of the concordance studies demonstrate that InnoQuant® HY produced quantification results in nearly 29% more than Quantifiler® Duo (indicating that this new kit is more effective in challenging samples), while the differences observed between InnoTyper® 21 and GlobalFiler™ are not significant. Therefore, the utility of InnoTyper® 21 has been proven, especially by the successful amplification of a greater number of complete genetic profiles (27 vs. 21). The results herein presented allowed the internal validation of both InnoQuant® HY and InnoTyper® 21, and their implementation in the LPC-PJ laboratory routine for the treatment of challenging samples.

1. Introduction

In forensic laboratories, new procedures, methods, kits, software or equipment need to be carefully evaluated and validated before its implementation [1]. Two types of validation exist: (i) developmental validation, performed by the manufacturer or a group of laboratories, with the objective to test new kits, primers sets or technologies for alleles detection; (ii) internal validation, more specific to the needs of a particular forensic laboratory, which consists of verifying that the established procedures previously examined by developmental validation will effectively work in the given laboratory [1,3].

According to the Scientific Working Group on DNA Analysis Methods (SWGDAM), the internal validation process should include five different studies: known and non-probative evidence samples, sensitivity and stochastic studies, precision and accuracy, mixture studies, and contamination assessment [4].

Known and non-probative evidence sample studies refer to methods proposed for casework samples that need to be evaluated and tested using known samples, non-probative evidence samples or mock case samples, and, when possible, authentic case samples. Results from these studies must be compared to the previous results of known samples and non-probative evidence or mock case samples to guarantee concordance [4].

Sensitivity and stochastic studies are used to demonstrate sensitivity levels of the test. As such, by testing a range of DNA

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concentrations, these studies estimate the dynamic range, ideal target range, limit of detection, limit of quantitation, heterozygote balance (e.g., peak height ratio), and the signal to noise ratio associated with the assay. Sensitivity studies may also be used to detect stochastic effects (stochastic threshold) usually resulting from low-quantity and low-quality samples [4,5].

Precision and accuracy are demonstrated by repeatability and reproducibility tests. Reproducibility tests are used to evaluate the average variation obtained by different operators using the same equipment to measure repeatedly the same sample. Repeatability tests are used to evaluate the variation of the measures obtained by a single operator, using the same equipment and method, to measure repeatedly the same sample [4,6].

Mixture studies are conducted to help forensic laboratories to establish guidelines for the interpretation of mixed DNA samples. These guidelines include determination of the number of contributors to a biological mixture, determination of the major and minor contributor profiles, and the proportions of each contributor in the mixed samples [4,5].

Finally, contamination assessment is performed using negative controls as well as known samples, to detect exogenous DNA which may be originated from reagents, consumables, operator and laboratory environment [4,5].

In addition, the European Network of Forensic Sciences Institutes (ENFSI) also proposes the inclusion of concordance studies where the same DNA samples are tested with different kits to verify if the results obtained are consistent between the kits. These studies are important to locate potential primer binding site mutations that could lead to allele drop-out [7,8].

InnoQuant® HY (quantification kit) and InnoTyper® 21 (amplification kit) are new commercial kits for DNA analysis that use retrotransposons as markers. Retrotransposons are class 1 Transposable Elements (TE) that resort to a copy-and-paste mechanism for its mobilization, constituting more than 40% of the human genome [9,10]. The mobilization mechanism resorts to a RNA intermediate which is then reverse transcribed into a complementary DNA (cDNA) copy by a mechanism called target-primed reverse transcription (TPRT), and then inserted into new genomics locations [11,12].

InnoQuant® HY is a real-time PCR system (qPCR) that allows evaluating both the quantity and quality of human DNA present in biological samples [13]. This kit was developed to detect total human and male DNA and uses two independent genomic targets – a short length multi-copy sequence (from an *Alu* element) and a long multi-copy sequence (from SVA element) – to qualitatively measure the degradation of a sample [2,13–15]. For the development of this multiplex four independent targets were used to design the primers and the TaqMan probes: (i) a short target from an *Alu* element (80 bp); (ii) a long target from a SVA element (207 bp); (iii) a male-specific target (79 bp); and (iv) an amplicon from a synthetic template (172 bp) used as Internal Positive Control (IPC) to detect PCR inhibition [2,13–14,16].

InnoTyper® 21 kit is a multiplex system based on *Alu* elements to determine small amplicon fragments (60–125 bp). It is compatible with existing PCR and capillary electrophoresis platforms, being particularly adequate for DNA typing of highly degraded and low concentration samples [17]. This multiplex consists of 21 genetic markers, including 20 retrotransposons and Amelogenin [15,20]. This selection, based on molecular characteristics and population data [18], includes highly polymorphic genetic markers (i.e., reaching 50% heterozygosity) from all major populations [17,19–20].

The aim of the present study was to internally validate the kits InnoQuant® HY and InnoTyper® 21 for implementation in the LPC-PJ laboratory routine. To this end, sensitivity, repeatability, and reproducibility parameters, as well as mixtures studies and concordance studies were evaluated.

2. Materials and methods

2.1. Sample Selection

For the sensitivity, repeatability, reproducibility, and mixture studies performed to validate the InnoQuant® HY and InnoTyper® 21 internally, the *InnoQuant® HY DNA Standard* and the *InnoTyper® 21 DNA Control*, respectively, were used.

For the concordance study, extracts of LPC-PJ casework (such as hairs, blood, contact trace, bones fragments, and teeth) were chosen, based on quantification values and the type of genetic profile previously obtained with Quantifiler® Duo and GlobalFiler™ tagged as “no results”, “inconclusive”, “complete”, and/or “possible degradation/inhibition” (Table 1).

2.2. DNA quantification and assessment of DNA degradation

DNA quantification was performed with InnoQuant® HY, according to manufacturer's instructions. The extent of DNA degradation in each sample was calculated using the ratio between the short and the long targets (DI). A DI of 1 indicates no degradation while a DI of 10 or more corresponds to significant degradation. InnoQuant® HY allows calculating the DI of samples, by the ratio between the concentrations of long and short targets as presented in the following equation:

$$\mathrm{DI} = \frac{[Short]}{[Long]} \quad (1)$$

2.3. PCR, electrophoretic conditions and genetic analysis

DNA amplification was performed using InnoTyper® 21, according to manufacturer's instructions. After amplification, samples were injected into the automatic sequencer 3130XL Genetic Analyzer (Applied Biosystems), where the amplified products were separated and detected by capillary electrophoresis. The results produced by the capillary electrophoresis instrument (electropherograms) were analysed using GeneMapper® ID-X v1.4 Software, with a minimum analytical threshold of 75 RFU.

2.4. Internal validation procedures

The parameters used for InnoQuant® HY and InnoTyper® 21 internal validation, were the minimum required by the ENFSI and included tests for sensitivity, repeatability, reproducibility, and mixture studies. Additionally, a concordance study between the results obtained from non-probative samples with the STR kits currently used by LPC-PJ (Quantifiler® Duo and GlobalFiler™) and the results obtained by these new kits were compared. During the validation, two different methodologies (manual and automatic) were used. The automatic methodology refers to quantitation set-up being performed by Qiagen® QIAgility™ robot to evaluate the possibility of automatization of these kits and the results obtained by both methodologies enabled the verification of the reproducibility of the kits. Quantification was performed in an ABI 7500 HID Real-Time PCR System and amplified fragments were run on the 3130XL Genetic Analyzer.

2.5. Sensitivity

Sensitivity testing for InnoQuant® HY was performed using a series of six dilutions of *InnoQuant® HY DNA standard* (from 1 to