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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

Table 1
Selected samples and their division according to the quantification value and genetic profile obtained with Quantifiler® Duo (QD) and GlobalFiler™ kits.

Group		Sample	Extract	Trace	Quantification value ng/μL (QD)	Profiling result (STR)
I		1	430-11	Hair	–	No results
		2	442-02	Hair	–	
		3	442-07	Hair	–	
II		40	442-01	Hair	–	Inconclusive
		42	358-03	Hair	0.003	
		43	442-04	Hair	–	
III		56	430-04	Hair	0.001	Complete
		57	447-08	Hair	0.024	
		58	442-09	Hair	0.087	
IV	IPC+	64	452-12	Biologic	–	No results
		65	454-06	Biologic	–	No results
		66	454-06	Biologic	–	No results
V	Degraded DNA	84	465-02	RS	0.006	Inconclusive
		85	457-17	RS	0.006	
		86	457-03	RS	0.010	
		87	447-05	Hair	0.011	
		88	375-24	RS	0.012	
VI	Degraded or inhibited DNA	113	101-01	Teeth	–	No results
		114	113-02	Teeth	–	No results
		115	113-01	Teeth	0.001	No results
		116	113-03	Teeth	0.260	Complete
		118	126-04	Teeth	0.008	Inconclusive
		119	122-03	Teeth	–	No results

0.03125 ng/μL), tested in duplicate. For InnoTyper® 21, the amplification of a series of five positive controls with different input volumes (5 μL, 4 μL, 3 μL, 2 μL, 1 μL) with concentrations ranging from 6.0 ng/μL to 0.12 ng/μL, prepared from *InnoTyper® 21 DNA Control* was performed and tested in duplicates.

2.6. Repeatability and reproducibility

For InnoQuant® HY, repeatability and reproducibility tests were based on the analysis of the C_T values obtained for the five standards used to create a calibration curve. These five standards were performed in duplicate and analysed in four different quantification runs thus obtaining eight C_T values for each standard in all the four quantification runs. Three of these quantification plates were performed by the QIAgility™ robot, at different times, and the fourth run was manually prepared. Thus, the same standards were prepared and run at different times and on different days, making it possible to evaluate the repeatability of the kit. For InnoTyper® 21, these tests were based on the amplification of the same series of five positive controls used in sensitivity tests, with different injection times [16 s, 18 s (default), and 20 s] and tested in duplicates.

2.7. Mixture studies

This parameter was only evaluated for the validation of InnoQuant® HY since the InnoTyper® 21 presents limitations in the interpretation of mixed DNA samples using conventional capillary electrophoresis. This study was performed by preparing mixtures from a male and a female sample (5.68 and 4.17 ng/μL, respectively), adjusted to a final concentration of 0.5 ng/μL, diluting the initial extract in TE. The preparation of mixture set was performed (Table 2) to test the ability of InnoQuant® HY to determine the proportions of the two contributors in these mixtures.

2.8. Concordance and non-probative sample study

For the concordance study, results from 132 samples – 129 casework samples with quantification results not concordant with the genetic profiles produced [e.g., sample with a quantification value of 0.265 ng/μL and an inconclusive genetic profile result (when a complete profile would be expected by the amount of DNA only, which can indicate degradation or the presence of PCR inhibitors)] and three control samples (DNA standard) - obtained with Quantifiler® Duo (quantification kit) and GlobalFiler™ (amplification kit), the STRs-based kits currently in use at LPC-PJ, were compared with the results obtained with the retrotransposons-based kits. As such, quantification results obtained with InnoQuant® HY were compared with those previously obtained with Quantifiler® Duo; while the genetic profiles produced using InnoTyper® 21 were compared with those previously obtained with GlobalFiler™.

2.9. Statistical analysis

Data were statistically analysed using Microsoft Excel 2010. T-student and Chi-square tests were performed to establish the significance of the results. P values of 0.05 or lower were considered as statistically significant. Results were expressed as the mean ± S.E.M. of the indicated number of experiments.

Table 2
Volume of each diluted DNA sample required to prepare the mixture set.

Mixture ratio							
	19:1	9:1	3:1	1:1	1:3	1:9	1:19
Female sample	95 μL	90 μL	75 μL	50 μL	25 μL	10 μL	5 μL
Male sample	5 μL	10 μL	25 μL	50 μL	75 μL	90 μL	95 μL

3. Results and discussion

The DNA quantification step of a given sample is of critical importance in forensic analysis (e.g., in criminal investigation and missing person identification) as it determines the most suitable amplification method to be implemented. Knowing the best amplification kit to be used and the optimal volume of DNA required for the run, allows saving time and financial resources. Determining the genetic profile of an individual in a given sample is the purpose of forensic genetics, whether in a criminal investigation or missing person identification. For this, amplification methods of the DNA extracted from these samples is a crucial step and, as such, the most important. As the DNA from forensic samples typically has low quality and quantity, there is the need to implement an amplification method that overcomes these problems. Thus, the development of a kit with the ability to amplify fragments as small as 125 bp becomes an advantage for forensic analysis.

3.1. Sensitivity

In which concerns the InnoQuant® HY, using the manual and automated (QIAgility™ robot) methods for sample preparation, the concentrations obtained for the serial dilutions were similar to the expected theoretical concentrations. Also, the quantification kit was able to quantify samples containing both high and low DNA concentrations, which indicates a good sensitivity (Table 3 and Table S1). When comparing the observed values to the expected values, the highest percent difference observed was for the first repetition of the 0.5 ng/μL dilution (26.04%), indicating a highly reproducible system even at the lowest concentration ranges tested.

For InnoTyper® 21, the optimal DNA quantity is within the 0.2–0.5 ng/μL interval. Therefore, the aim of the sensitivity test was to evaluate the ability of the kit to produce good quality genetic profiles outside the optimal range. The results showed that this kit was able to amplify DNA quantities above and below the optimal DNA template target range though. When the DNA concentration was below 0.2 ng/μL, the percentage of amplified alleles was considerably reduced. Also, a small difference between the first and the second repetition performed was observed (Table 4 and Table S2), but it is unlike to compromise the results ($p > 0.05$). Concerning the concentrations within optimal amplification range, the results differed from the expected values since complete profiles were not obtained. However, the number of amplified alleles was similar to the entire profile, and an amplification failure of the same marker in the positive controls 2–4 was observed. These small discrepancies observed were probably related to amplification problems (Table 4). In general, the results obtained validate the sensitivity of the InnoTyper® 21, to amplify samples with low concentrations and concentrations above the optimal amplification range.

3.2. Repeatability and reproducibility

Concerning InnoQuant® HY repeatability and reproducibility, each standard was applied in duplicate in each run, producing six C_T values (C_T1 to C_T6) in the set of three automated runs. To perform the fourth run, the same dilutions were manually prepared and applied, in duplicate, producing two more C_T values: C_T7 and C_T8. In the same run, no statistically significant differences of C_T values between duplicates were observed, as well as among the different standards ($p > 0.05$). The same was registered for the fourth run, except for the standards 1 (20 ng/μL) and 5 (0.005 ng/μL). In these cases, the difference between duplicates did not compromise the results (Fig. 1 and Table S3), and it may have resulted of the fact that these standards contained lower (standard 5) and higher concentrations (standard 1).

As expected, as the concentration of DNA standards decreased, an increase of the C_T values was detected. The same pattern, an inverse proportionality between the concentration values of the DNA standards and the CT values was observed in all runs, which demonstrates the high consistency of the kit (Fig. 1 and Table S3).

By comparing the C_T values obtained for the three quantification runs prepared by the robot (C_T1 to C_T6) with the C_T values in the manual run (C_T7 and C_T8), an increase in the C_T values for this last run was observed. This variability may be associated with a variation in the machine conditions since the runs were performed on different days, by different operators, as well as the fact that the preparation of the standards for the manual run was performed from a new *InnoQuant® DNA HY Standard* solution. However, it is important to note that these changes observed for the C_T values between runs were not significant ($p > 0.05$) (Table S3) and, therefore, did not affect the kit reproducibility, since all values were within the optimal range of 30 C_T stipulated by manufacturers.

For each of the three quantification runs prepared by the robot, a calibration curve for DNA concentration was obtained, using the C_T average values from each standard. The calibration curve was also determined for the set of the runs (Av. run), considering the average values of total C_T obtained for each of the five standards (Fig. 2). The calibration curve as a function of the logarithm DNA concentration was also determined in the fourth quantification run (Fig. 3). In all calibration curves, a linear regression with negative

Table 3
Quantification values and percent differences from the expected values obtained with the short target of InnoQuant® HY (manual and QIAgility™ robot procedures). Conditional formatting was applied to the percent difference columns, with the darkest cells indicating the highest values. (For interpretation of the references to colour in this Table legend, the reader is referred to the web version of this article.)

Dilution	Expected values (ng/μL)	Observed values (ng/μL) - QIAgility™ robot				Observed values (ng/μL) - Manual	
		1st repetition	% Difference	2nd repetition	% Difference	1st repetition	% Difference
Intermediate	10	13.067	23.47	11.288	11.41	-	-
Dil.1	1	1.097	8.84	1.133	11.74	0.9	10.00
Dil.2	0.5	0.676	26.04	0.601	16.81	0.463	7.40
Dil.3	0.25	0.284	11.97	0.299	16.39	0.215	14.00
Dil.4	0.125	0.143	12.59	0.141	11.35	0.124	0.80
Dil.5	0.063	0.064	1.56	0.071	11.27	0.056	11.11
Dil.6	0.031	0.034	8.82	0.038	18.42	0.033	6.06

Table 4
Number of amplified alleles and respective percentage for each of the positive controls analysed.

Samples	Concentration (ng/μL)	Obtained alleles (n)		Obtained alleles (%)	
		1st repetition	2nd repetition	1st repetition	2nd repetition
PC1	0.12	34	28	80.95	66.67
PC2	0.24	40	40	95.24	95.24
PC3	0.36	40	41	95.24	97.62
PC4	0.48	42	41	100	97.62
PC5	0.60	42	42	100	100
NTC	0	0	0	0	0

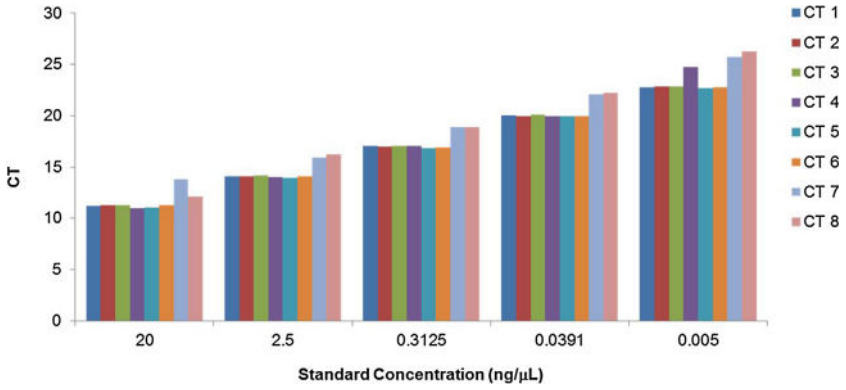

Fig. 1. C_T values obtained for the short target of five standards during the three quantification runs.

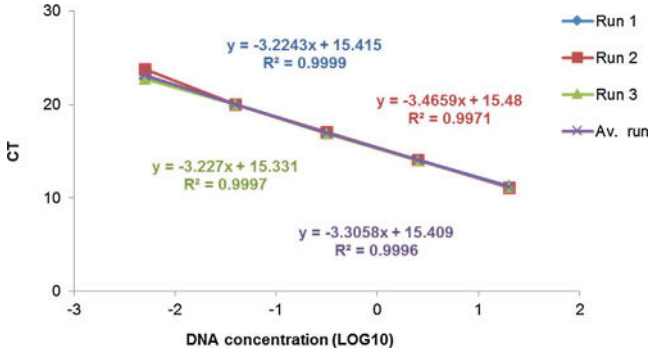

Fig. 2. Calibration curves for the quantification runs performed by the robot as well as for the average run (Av. run).

slope was observed showing the existence of inverse proportionality between the C_T values and the DNA concentration. All calibration curves, including the calibration curve for the set of the three runs (Av. run), were similar, substantially overlapping with each other with the exception of run 4 that although similar, was parallel to the others. All curves presented correlation coefficients (R^2) higher than 0.99, demonstrating primer efficiency and showing the proximity between the regression line and the C_T values for each standard. Thus, the consistency of results for the C_T values of each of the analysed standards, demonstrated the kit's repeatability and reproducibility.

The study of InnoTyper® 21 repeatability and reproducibility was performed by the analysis of the genetic profiles obtained for the five positive controls (PC1 to PC5) used during the sensitivity test. Considering the results for the duplicates within each injection time, a similar percentage of amplified alleles for each control was obtained (Fig. 4). Also, the results demonstrated a large percentage of amplified alleles for all the controls (>94%) except for the PC1 whose percentage of amplified fragments was lower (64–74%) but not significant ($p > 0.05$) (Fig. 4 and Table S4). The

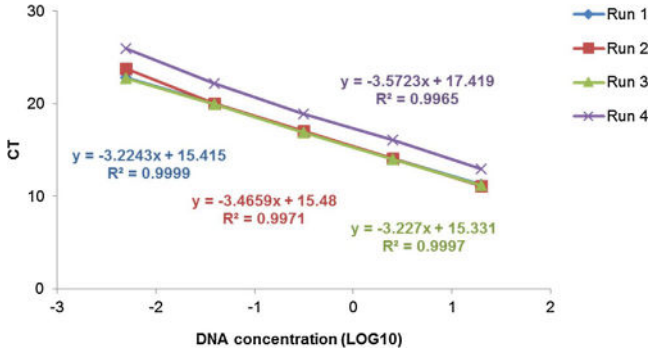

Fig. 3. Calibration curve for all four quantification runs.

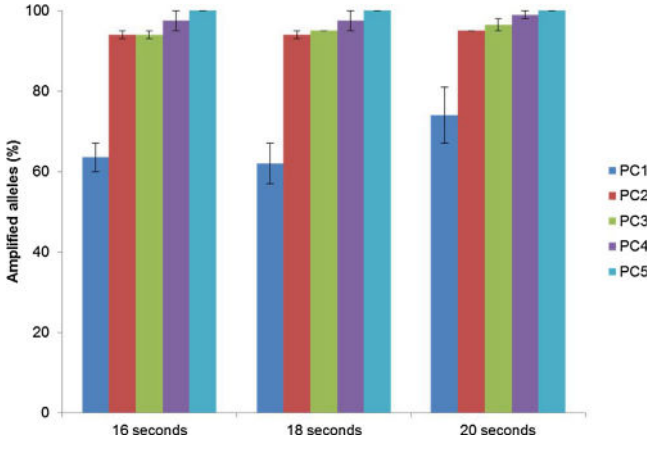

Fig. 4. Percentage of amplified alleles for each positive control run in replicate with three different injection times. Data are mean ± sd of two experiments.

discrepancy observed was probably associated with the low DNA concentration in this sample.

Overall, in the three runs performed, the percentage of amplified alleles for each of the five controls was similar. Thus, the genetic profiles obtained prove the repeatability of InnoTyper® 21, as well as its reproducibility, allowing the internal validation of this amplification kit.

3.3. Mixture studies

The ability to quantify different concentrations of male and female DNA in mixtures was evaluated by the analysis of quantification results for each M:F ratio. The concentration values of total DNA and male DNA resulted from the quantification results obtained for the short and Y targets, respectively. The concentration of female DNA is an approximate value, resulting from the subtraction of the male DNA concentration from the total DNA concentration (Table 5).

The analysis of the quantification values obtained for both male and female DNA, it was possible to determine (even at the highest ratio tested) the major and minor contributors for each sample, attesting the kit's ability to discriminate between two different contributors with different proportions. Thus, the results obtained for this parameter demonstrated the ability of InnoQuant® HY to quantify different concentrations of male and female DNA in mixtures with different M:F ratios, and most importantly, to detect the presence of low levels of male DNA in the presence of high levels of female DNA.

3.4. Concordance and non-probative samples study

In the context of forensic analysis, a concordance study was performed to evaluate the performance of InnoQuant® HY in challenging samples, in comparison with Quantifiler® Duo. Among the 132 samples analysed, three presented no quantification values for any of the kits tested, due to the absence of DNA, as a result of a less accomplished extraction. Approximately 96 % and 93 % (Short and Long targets, respectively) of the casework samples were successfully quantified with InnoQuant® HY. Concomitantly, with Quantifiler® Duo autosomal target, quantification values were only obtained in 64 % of the samples (Fig. 5).

The comparison between the long target of InnoQuant® HY and the human target of Quantifiler® Duo, demonstrated that InnoQuant® HY was more effective in analysing challenging samples, since this kit was able to produce quantification results in 29 % more samples than with Quantifiler® Duo.

The DI was calculated for all the samples analysed (Table 6). However, only 123 of the samples presents DI results (five samples did not present quantification values for none of the analysed targets – short or long – and four samples did not present quantification values for the long target, which limited DI calculation). A significant percentage of the samples (77 %) presented moderate ($3 < DI < 10$; 46 %) to high degradation ($DI > 10$; 31 %). High degradation values can cause problems in assessing the amount of DNA input for amplification, leading to subsequent rework. A subset of 28 samples presented low degradation ($DI < 3$; 23 %). Among these, only 13 presented $DI = 1$ (not degraded), being distributed by the sub-groups II, III, IV and VI. It was noted that for the samples with a $DI < 3$, all the samples with quantification values superior to 0.1 ng/μL for both quantification kits (Quantifiler® Duo and InnoQuant® HY), presented a full profile. Also, it was verified that for the samples exhibiting moderate to high degradation, presenting quantification values greater than 0.1 ng/μL, most of the full profiles were obtained when the amplification was performed with InnoTyper® 21. This indicates sufficient degradation to cause issues in obtaining optimal DNA STR typing results. Thus, this new feature provided by InnoQuant® HY allowed a prior knowledge about the DNA quality present in the samples and to determine the best strategy to be adopted for results production, minimizing the need for unnecessary re-amplifications.

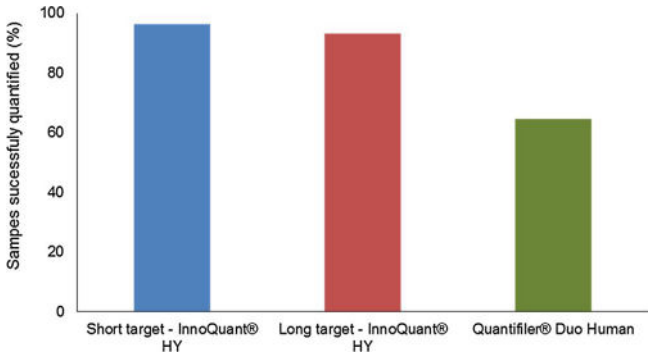

Fig. 5. Percentage of quantified samples for each target of InnoQuant® HY and Quantifiler® Duo (n = 132).

As described for the quantification kit, this study was also performed to evaluate the performance of InnoTyper® 21 in challenging samples for forensic analysis when compared with the GlobalFiler™. Therefore, from the 132 samples previously quantified with InnoQuant® HY, 127 were selected for the subsequent amplification with InnoTyper® 21, using a DNA input of 0.5 ng/μL.

Comparing the results obtained with the two kits, it was observed that both presented difficulties in the amplification of the selected samples, and with this new amplification kit nearly 9 % of the samples produced no results. In addition, InnoTyper® 21 produced more complete genetic profiles (27 vs. 21 obtained with GlobalFiler™) (Fig. 6). However, it should be noticed that the GlobalFiler™ amplification was performed using fresh extracts, whereas for InnoTyper® 21 the selected extracts were previously subjected to several freezing-thawing cycles. Therefore, these cycles can be the determining factor to explain the difference between the performances of the two kits compared in this study.

Overall, the results herein presented demonstrated that for challenging samples and under the conditions used in this study, the differences observed between the two kits were not significant.

Table 5
Quantification results obtained for the different male:female (M:F) ratios from mixtures.

M:F ratio expected (ng/μL)	Total DNA (ng/μL)	Male DNA (ng/μL)	Female DNA (ng/μL)	M:F ratio obtained (ng/μL)
19:1	0.182	0.175	0.006	25:1
9:1	0.168	0.148	0.020	7.40:1
3:1	0.188	0.128	0.060	2.13:1
1:1	0.180	0.083	0.097	1:1.17
1:3	0.215	0.051	0.163	1:3.13
1:9	0.266	0.030	0.235	1:7.83
1:19	0.227	0.014	0.213	1:15.21

Table 6
Degradation index in the set of analysed samples (QDuo = Quantifiler® Duo; GF = GlobalFiler™; IQHY = InnoQuant® HY; IT = InnoTyper® 21).

DI	Number of samples	%	Number of samples with QDuo > 0.1 ng/μL	Full profile with GF	%	Number of samples with IQHY > 0.1 ng/μL	Full profile with IT	%
<3	28	22.8	8	8	100	11	11	100
3–5	33	26.8	1	0	0	2	1	50
6–10	24	19.5	2	1	50	2	2	100
11–15	6	4.9	0	0	0	1	1	100
16–20	6	4.9	0	0	0	0	0	0
21–30	9	7.3	0	0	0	0	0	0
31–60	6	4.9	0	0	0	0	0	0
>60	11	8.9	0	0	0	1	1	100

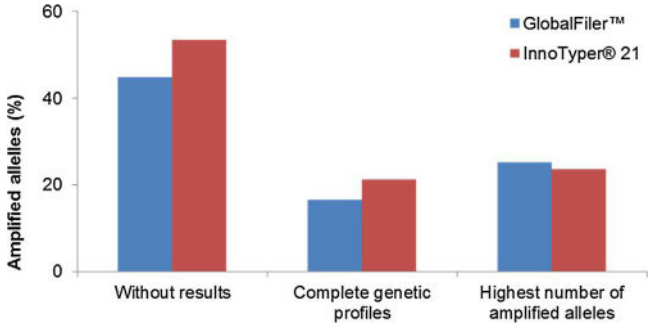

Fig. 6. Amplified alleles from the samples analysed with InnoTyper® 21 and GlobalFiler ™ (n = 127).

However, it can be hypothesized that the fact that InnoTyper® 21 was operated with extracts subjected to freezing and thawing cycles may have impaired the results produced by this kit (the results produced by GlobalFiler™ were obtained from fresh extracts). Thus, the results obtained in this study (mostly those presented in Table 6), demonstrated that InnoTyper® 21 produce more amplified alleles for the more highly degraded samples when compared with Globalfiler™. Therefore, the utility of InnoTyper® 21 has been proven, and an especial focus shall be given to the successful amplification of a greater number of complete genetic profiles.

4. Conclusion

The results herein presented for the internal validation procedure for InnoQuant® HY and InnoTyper® 21 demonstrated the sensitivity, repeatability, and reproducibility, and discriminatory resolution in mixture samples (M:F) of these kits. Validation studies were conducted for both kits according to the guidelines established by SWGDAM [4] and ENFSI [6]. Therefore, the efficiency of both kits was proven allowing its implementation in the routine laboratory at the LPC-PJ for the analyses of challenging samples.

Declaration of interest

All authors disclose any actual or potential conflict of interest. InnoGenomics Technologies has not interfered in whatsoever with the research and simply provided the kits.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.forsciint.2017.11.037.

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