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RESEARCH ARTICLE

Comparison and Internal Validation of GlobalFiler™, VeriFiler™, and Investigation® 24Plex PCR Kits in Forensic DNA Identification

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ABSTRACT

In the forensic biology laboratory, an internal validation process is performed to determine the reliability and reproducibility of an identification kit. STR kits, which are used for identification with DNA are commercially produced by various companies. Due to primary design differences by the companies, there may be differences in the performance of STR loci in the kits depending on the devices used in the laboratory. The main purpose of this study is to determine which kits are the most ideal and reliable in terms of reproducibility and accuracy of results. In this study, positive control samples included in the kit and fingertip blood, hair and buccal swab samples obtained from 4 volunteer laboratory personnel were used. After DNA isolation and quantification, PCR amplification was performed with GlobalFiler™, VeriFiler™ Plus and Investigator® 24plex QS PCR Amplification Kit. Amplicons were electrophoresed in 3130xl Genetic Analyzer and analyzed with GeneMapper ID-X v1.5. DNA materials from blood, hair and buccal swap samples of volunteers were tested. Full analyzable profiles were obtained with 1-0.25 ng/µl. Drop outs were observed in the alleles obtained with 0.0625 ng/µl DNA. The results obtained from these kits were complementary to each other. Optimization studies are planned for future studies on forensic DNA identification analysis from crime scene samples such as bones, teeth and semen.

INTRODUCTION

Identification studies are carried out preferably at the DNA level in solving criminal cases, identifying victims of disaster, terrorist attacks and lineage determination (DVI, 2007). Repeated sequences located in the non-coding gene region of DNA and showing polymorphic properties are known as STR regions. For this purpose, STR regions that are located in non-coding DNA regions are analyzed easily and showing high reliability, are routinely applied in forensic DNA identification. The base count of repeating units in STRs is lower than in minisatellites, therefore they are called microsatellites. Microsatellites or STRs(Short Tandem Repeats) are simple sequences, widely found in many eukaryotic genomes. Microsatellites are formed with 2-7 base pairs units repeated 5-25 times. They can be amplified easily because they are shorter than 100 bp. Utilization of STR gene regions and number of STR loci in the studies have been rapidly increasing day by day since 1985 (Butler, 2012). Right now, 24 gene regions are utilized in the identification on DNA levels. However, in the beginning, only 3 gene regions were used (Butler, 2015). This technology gained momentum with the capillary electrophoresis method in 2000's and now is evolving into NGS (Next Generation Sequencing)

methods (Alvarez-Cubero et al. 2017). However, NGS is not yet widely preferred in routine inspections because of its high cost(Butler, 2015). Although it is thought that SNPs (Single Nucleotide Polymorphism) will replace STRs in the future, in terms of cost, reliability and accuracy, STRs do not seem to be easily replaced by SNPs (Butler, Coble, and Vallone 2007).

STR loci, which constitute the repetitive gene regions of DNA, are electrophoresed after being amplified by the PCR method. The peaks obtained from the analysis program are evaluated as a DNA profile (Butler 2015;Pankaj Shrivastava et al. 2020). Laboratory analysis is carried out by comparing known gene regions (reference samples) and unknown DNA samples (crime scene samples) belonging to the same STR locus. For evaluation purpose, allele comparison at each STR locus can be made using the str loci of crime scene and reference samples, without performing numerical and statistical calculations. For comparing and further developing a DNA database, each different laboratory around the world work with the same STR loci. Consequently, in 1997, a DNA database named CODIS (Combined DNA Index System) was established in the USA. There are 16 STR loci in CODIS that were determined by the FBI. These loci are as follows; D3S1358, vWA, FGA, D8S11179, D21S11, D18S51, D5S818, D13S317, D7S820, D16S539, THO1, TPOX, CSF1PO. The database system compares and evaluates barcodes obtained through DNA analysis(Hares 2012). Because of this database, comparisons can be made between the DNA profiles registered in the criminal laboratory in the network with the crime scene DNA profile and the DNA profile obtained from the biological samples of suspects or victims. The registry in the FBI laboratory gained recognition globally over time. CODIS DNA database, with mutual data sharing, is getting used quite extensively in criminal investigations. As time goes by, there are more countries sharing data with the database (Hares 2015).

The original CODIS database, which consist of 13 STR loci, was mostly successful in matching suspects' data to evidence. To strengthen the power of discernment, reduce the probability of random matching, and widen the global data-sharing network CODIS Loci Working Group decided to include three "highly recommended" loci (SE33, DY391, Amelogenin) to further increase the sensitivity of the study, in addition to the 20 STRs of the CODIS' main loci (Kreamer M et al. 2017; Tao et al., n.d.) This study was designed to be a pilot evaluation of this STR-PCR kit in a Chinese Han population regarding the PCR conditions, sensitivity, precision, accuracy, repeatability, reproducibility, and concordance; tolerance to PCR inhibitors; applicability to real "forensic-type" samples; species specificity; mixture, balance and stutter analyses, and utility in a population investigation. The exhaustive validation studies demonstrated that the Investigator 24plex QS system is accurate, sensitive, and robust for STR genotyping. In addition, these genetic markers in the population data in our study indicated that they can also be useful for forensic identification and paternity testing in the Chinese Han population. This study evaluated the following three forensic DNA identification devices regarding their performance and internal validation. These are; GlobalFiler™ PCR Amplification Kit (ThermoFisher), VeriFiler™Plus PCR Amplification Kit (ThermoFisher), and Investigator® 24plex QS PCR Amplification Kit (Qiagen).

GlobalFiler™ PCR Amplification (ThermoFisher) Kit includes; D13S317, D7S820, D5S818, CSF1PO, D1S1656, D12S391, D2S441, D10S1248, D18S51, FGA, D21S11, D8S1179, vWA, D16S539, TH01, D3S1358, Amelogenin, D2S1338, D19S433, DYS391, TPOX, D22S1045, SE33 and Yindel STR loci ("TFS-AssetsLSGmanuals4477604.Pdf," n.d.; Ludeman et al. 2018; Wang et al. 2015). In determining the loci, 6-fluorescently labeled TAZ™ (red), 6-FAM™(blue) NED™ (yellow), SID™ (purple), VIC™ (green), LIZ™ (orange) fluorochrome dyes were used in the PCR system. For internal validation Control DNA 007 (0.1 ng/µl) sample was utilized as a reference in the kit.

VeriFiler™ Plus PCR Amplification (ThermoFisher) Kit includes STR loci of; D3S1358, vWA, D16S539, CSF1PO, D6S1043, D8S1179, D21S11, D18S51, D5S818, D2S441, D19S433, FGA, D10S1248, D22S1045, D1S1656, D13S317, D7S820, Penta E, Penta D, TH01, D12S391, D2S1338, and

TPOX, two internal quality control markers (IQCS and IQCL), Y chromosome insertion/deletion polymorphic marker (Yindel) and sex marker (Amelogenin) (Green et al. 2021; Qu et al. 2024) and contains Control DNA 007 (0.1 ng/µl). In this system, 6-fluorochrome dyes were used marking: TAZ™ (red), 6-FAM™(blue), TED™(yellow), SID™ (purple), VIC™(green), LIZ™ (orange) VeriFiler™ Plus kit, which is thought to be more performant in terms of validation, includes all DNA loci that form important national and local databases including the 20 core loci recommended by Chinese Ministry of Public Security and CODIS, as well as highly discerning Penta D and Penta E loci (Zhong et al. 2019).

Investigator® 24plex QS PCR Amplification (Qiagen) Kit includes; D1S1656, D2S441, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D10S1248, D12S391, D13S317, D16S539, D18S51, D19S433, D21S11, D22S1045, CSF1PO, FGA [FIBRA], TH01 [TC11], TPOX, vWA, SE33 [ACTBP2] and DYS391, Amelogenin(sex marker) and quality censors (QS1 and QS2). Consisting STR loci are 6-FAM, BTG, BTY, BTR2, BTP, and BTO, which are fluorochrome multiplex loci. Control DNA 9948 (0.1 ng/µl) was used for reference in an internal validation study (Kreamer M et al. 2017; "Evaluation of the Investigator® 24plex GO! Kit and Associated Allele Frequency Data for Four South African Population Groups" 2024; Correa et al. 2019). This kit is specifically designed for the analysis of crime scene biological samples that are low in both quality and quantity. The PCR performance with samples containing trace amounts of DNA is consistent. By using the PCR control (Quality Sensor QS1 and QS2) included in the multiplex kit, PCR amplification quality, unsuccessful PCR amplification due to the absence of DNA, and PCR results that may result from degraded DNA can be monitored with quality sensors.

The performance evaluation and internal validation study of three PCR amplification kits were planned within the scope of TS EN ISO IEC 17025 accreditation criteria (ISO/IEC 17025:2017, n.d.) and SWGDAM criteria (Scientific Working Group, n.d.) which are essential for forensic biology laboratories. The study aims to determine the performance of PCR amplification kits that will be used for the first time in forensic DNA identification in our laboratory and to carry out internal validation based on accreditation criteria.

MATERIALS AND METHODS

DNA SAMPLES

DNA was isolated from fingertip blood (B), hair (H), and buccal swap (BS) samples belonging to volunteering 2 female and 2 male laboratory workers, using an EZ1 DNA Investigator kit ("EZ1 Advanced XL Robotic Platforms and DNA Intestigator Extraction Kit Validation Report.Pdf," n.d.) in EZ1® Advanced XL automatic isolation system. The quantity of the DNA in before mentioned samples was determined in the Real-Time PCR-BAX® System Q7 system with Quantifiler™ Trio DNA Quantification Kit (Applied Biosystems, Cat. No. 4482910) ("4344b0_813b241e8944497e99b9c45b163b76bd.Pdf," n.d.). In the study, Control DNA 007 (0.1ng/µl) and Control DNA 9948 (0.1ng/µl) samples were used for performance evaluation. Abbreviations for the biological samples belonging to four volunteering workers are as follows:

GG H (Hair, Female), GG B (Blood, Female), GG BS (Buccal Swab, Female)

AA H (Hair, Female), AA B (Blood, Female), AA BS (Buccal Swab, Female)

MM H (Hair, Male), MM B (Blood, Male), MM BS (Buccal Swab, Male)

OO H (Hair, Male), OO B (Blood, Male), OO BS (Buccal Swab, Male)

Each kit to be internally validated was studied according to the amplification protocol of the STR locus it contained.

PCR study with GlobalFiler™ PCR Amplification Kiti (ThermoFisher)

DNA isolates (GG H, GG B, GG BS, AA H, AA B, AA BS, MM H, MM B, MM BS, OO H, OO B, OO BS) were amplified using GlobalFiler™ PCR Amplification Kit (ThermoFisher) according to Ludeman et al.(Ludeman et al. 2018) method. In the PCR setup study, negative control and positive control (Control DNA 007 -0.1 ng/µl) included in the kit were used. GlobalFiler™ reaction components were prepared as 7.5 µl Master Mix and 2.5µl Primer Set for each reaction. DNA and Nuclease-free water were then added to the reactions total volume amounting to 25 µl. The PCR reaction was carried out in the Thermal Cycler (Bio-Rad® T-100) according to the profile description in Table 1. After amplification until the electrophoresis stage, PCR amplicons were evaluated using Applied Biosystems 3130xl Data Collection Software v.4 and GeneMapper ID-X v.1.5 software (Figure 1).

Table 1: Program the thermal cycling conditions for GlobalFiler™ PCR gene regions

Temperature	Time	Number of Cycles
95 °C	1 min	-
94 °C	10 sec	29 cycle
59 °C	90 sec	
60 °C	10 min	-
4 ºC	∞	-

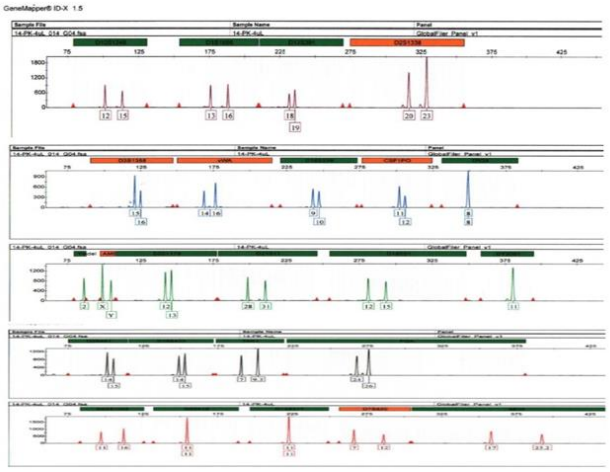

Figure.1: DNA Control 007 (0.1 ng/µl) amplified with the GlobalFiler™ PCR amplification kit and analyzed on an Applied Biosystems 3130xl Genetic Analyzer.

PCR study with VeriFiler™ Plus PCR Amplification Kiti (ThermoFisher)

DNA isolates (GG H, GG B, GG BS, AA H, AA B, AA BS, MM H, MM B, MM BS, OO H, OO B, OO BS), were amplified using VeriFiler™ Plus PCR Amplification Kit according to the protocols Green et al. (Green et al. 2021). VeriFiler™ Plus PCR reaction components were prepared as 5.0 µl Master Mix and 2.5 µl Primer Mix with 17,5 µl (0.5 ng/µl) sample DNA, amounting to 25 µl in total volume. Negative control and positive control (Control DNA 007 -0.1 ng/µl) were used in each study. The PCR reaction was carried out using a ThermalCycler (Bio-Rad® T-100) according to the cycle described in Table 2. Post-amplification PCR amplicons were evaluated using Applied Biosystems 3130xl Data Collection Software v.4 and GeneMapper ID-X v.1.5 software.

Table 2: Program the thermal cycling conditions for VeriFiler™ Plus gene regions

Temperature	Time	Number of Cycles
95 °C	1 min	-
96 °C 62 °C	10sec 90 sec	1 cycle
96 °C 59 °C	10 sec 90 sec	26 cycle
60 °C	5 min	
4 ºC	∞	-

PCR study Investigator® 24plex QS Kit

DNA isolates (GG H, GG B, GG BS, AA H, AA B, AA BS, MM H, MM B, MM BS, OO H, OO B, OO BS), were amplified using Investigator® 24plex QS PCR Kit according to Kreamer et al. (Kreamer M et al. 2017) 17,5 µl of 0.5 ng/µl DNA and reaction components of 7,5 µl were prepared according to Table 3. In each study negative and positive controls (Control DNA 9948) were used. The Master Mix prepared in this way was distributed in 10 µl into 0.2 µl tubes. Sample DNA and Nuclease-free water were added to make the total volume 25µl. The PCR reaction was performed in a Thermal Cycler (Bio-Rad® T-100) according to the cycle described in Table 4. Post-amplification PCR amplicons were evaluated using Applied Biosystems 3130xl Data Collection Software v.4 and GeneMapper ID-X v.1.5 software according to the allelic ladder(Figure 2).

Table 3: Reaction setup of Investigatior® 24Plex QS kit

Components	Volume per reaction
Fast Reaction Mix 2.0	7.5µl
Primer Mix Nuclease Free Water Sample DNA	2.5µl Variable Variable
Total Volume	**25µl**

Table 4: Program the thermal cycling conditions for Investigator 24plex PCR gene regions

Temperature	Time	Number of Cycles
98 °C 64 °C 72 °C	30sec 55sec 5sec	3 cycle
96 °C 62 °C	10sec 90sec	27 cycle
68 °C 60 °C	5min 5min	- -
10 °C	∞	
4 ºC	∞	

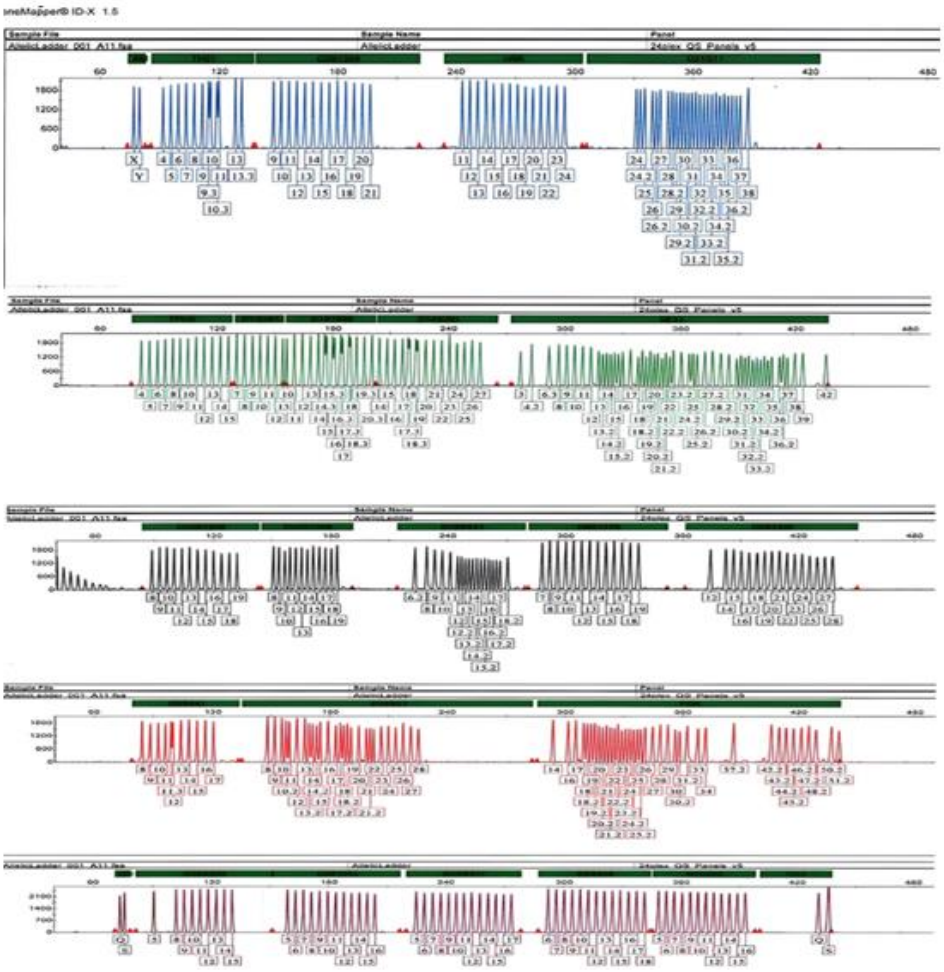

Figure 2: Electropherogram of the allelic ladder Investigator® 24Plex analyzed on an Applied Biosystems 3130xl Genetic Analyzer.

RESULTS

Sensitivity Study, SWGDAM guideline 3.3 recommendations

Control DNA 007 reference sample (1 ng/µl) of GlobalFiler™, VeriFiler™Plus and Investigator® 24plex PCR Amplification Kits were amplified in duplicate within the range of 1-0.312 ng/µl at different concentrations (1, 0.5, 0.25, 0.125, 0.625, 0.0312) according to the described cycle in Table 1. Negative control was used in each study. No contamination was observed. By amplifying 1-0.5 ng/µl DNA in 29 cycle GlobalFiler™ PCR amplification kit (Table 5), full profile peaks were obtained in all gene regions. 0.5 ng/µl DNA was amplified in 30 cycles with the Investigator 24 Plex PCR amplification kit protocol. Full profile peaks were detected in all gene regions. It was determined that PCR amplification showed quality performance with the two internal PCR controls (QS1 and QS2) included in the kit. Reliable and stable peaks were seen in 82 percent up to a concentration of 0.125 ng/µl. Full profile results could be analyzed in amounts of DNA greater than 0.1 ng/µl. In the same study, full profile peaks were obtained as a result of 0.5 ng/µl DNA in 27 cycle with two female and male hair samples (AA H, MM-H, GG H, OO H). Negative control was also studied and no contamination was observed.

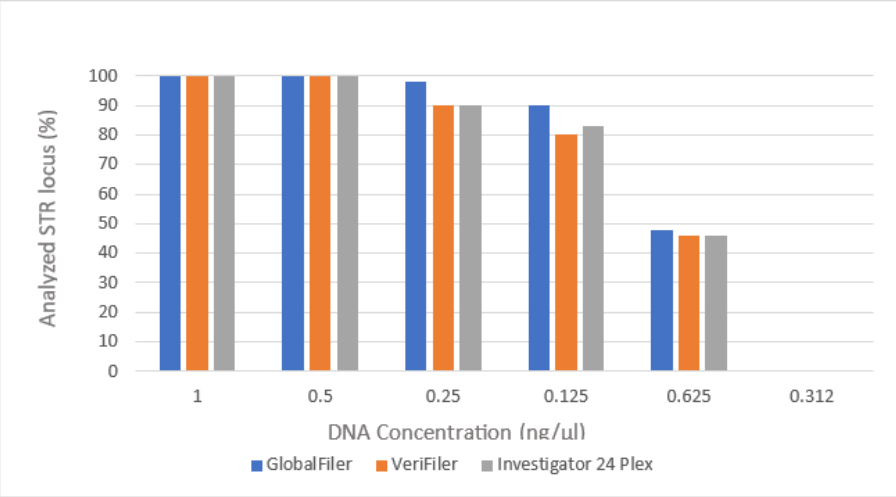

Figure 3: Comparison of sensitivity between GlobalFiler™, VeriFiler™ and Investigator® 24plex Kits.

In Control 007 peaks could be analyzed at a concentration between 0.5-0.0312 ng/µl. At 0.0312 ng/µl, allele dropout was detected in 15 regions, and peak imbalance was detected in 18 regions. Peak imbalances were detected in 12 regions at 0.0625 ng/µl; in 4 regions at 0.125 ng/µl; and in 6 regions at 0.25 ng/µl. The ideal DNA concentration was determined as 0.5 ng/µl. The samples to be amplified with the GlobalFiler PCR amplification kit were electrophoresed on 3130xl genetic analyzer (ThermoFisher) with Windows™ 7 DataCollection software v.4 installed in the HID-FragmentAnalysis36_POP4_1, Injection conditions: 15kV/1500sec, Dye Set J6, run module. The results obtained were compared according to the STR loci corresponding to allelic ladder in each kit.

Repeatability Study, SWGDAM guideline 3.5.1 recommendations

PCR studies were performed with GlobalFiler™, VeriFiler™Plus, and Investigator® 24plex QS PCR Amplification Kits according to their protocols (Elwick K, Mayes C, and Hughes-Stamm, S 2018; Scientific Working Group, n.d.; "ENFSI Best Practice Manual December 2022 Human Forensic Biology and DNA Profiling,"n.d.). Technical staff in the laboratory also participated in the study as working technicians. PCR amplification of each kit was performed at different times. Samples were loaded into the devices at different times (within 3 days). The obtained profiles were compared with positive control samples (Control DNA 007 ve 9948M). The study was repeated in groups of two. In this study, VeriFiler™ Plus and Investigator® 24plex PCR amplification kits were studied. The gene regions contained in each kit were compared with the alleles determined in the STR loci in each kit. Verification was made between the PCR results worked by technical staff. In the repeat studies, no difference was detected in the results depending on the personal working (Figure 1).

Reproducibility, SWGDAM guideline 3.5.2 recommendations

In this study, the results of GlobalFiler™ VeriFiler™Plus and Investigator® 24plex PCR amplification gene regions were evaluated both quantity-wise (numerically) and in terms of peak balance and height. Accuracy and precision of test performed according to SWGDAM guideline 3.5.2 criterion; it was tested in duplicate studies with different employees and different DNA samples. It was determined that the same PCR result was obtained from the same sample even though different technicians worked. The results in the gene regions constituted content of the PCR kits were evaluated both numerically and by comparing the peak balance and height with the criteria defined in the amplification kit guide and taking into account the average deviations of each locus. It was determined that the heights of the peaks and the frequency of drop-in and drop-out occurred after 0.0625 ng/µl depending on the amount of DNA. The quality was evaluated by looking at the peak

heights of the STR loci in the kits and the RFU values of the peaks in the DNA profiles. Precision, repeatability, reproducibility, stochastic threshold, and quality control studies were carried out within the scope of SWGDAM criteria. Analyzable full profiles were obtained with 1-0.25 ng/µl DNA. Allele losses (drop-out) were observed in the alleles obtained with 0.0625 ng/µl DNA. For his study dilutions at different concentrations were created from the reference sample (Control DNA 0.1-0.312 ng/µl). Each sample was prepared in duplicate. These transcripts were amplified with GlobalFiler, VeriFiler, and Investigation 24plex PCR amplification kits. Ideal injection and voltage parameters for electrophoresis of each kit were determined on the 3130xl. Full profile DNA was obtained in the alleles of STR loci obtained from the volunteers' buccal swap, hair and blood samples, and reference control DNA samples (1.0-0.25 ng/µl).

Mixture DNA Study (SWGDAM guideline 3.8 recommendations)

The mixture study was prepared from four samples that the amounts of DNA are known. Mix DNA samples of two and three individuals were prepared in a 1:1 ratio, the first being female and male, and the second being one female and two male DNA (GG H+ OO H + MM H) (Table 6). The amounts of DNA ("ENFSI-Validation-Guideline-04012024.Pdf," n.d.; "Best Practice Manual for the Internal Validation of Probabilistic Software to Undertake DNA Mixture Interpretation 2012," n.d.) added to the mixture were between 0.09-0.16 ng/µl.

Table 5: Mixture samples

Sample Name	Sample type	Mix ratio
AA H + MM H	Hair	1:1
GG-H+ OO H + MM H	Hair	1:1:1

These amounts were used to determine the lowest amount at which the allele contributing least to the mixture could be distinguished. After DNA samples were amplified separately for each kit, they were electrophoresed on a 3130xl genetic analyzer and analyzed with GeneMapper ID-X v.1.5 software. The evaluation was made according to ENFSI's Best Practice Manual("ENFSI Best Practice Manual December 2022 Human Forensic Biology and DNA Profiling," n.d.) criteria. Internal validation studies were carried out with both control DNA samples and biological samples, taking into account ENFSI and SWGDAM recommendations (Scientific Working Group, n.d.). Major and minor alleles were determined separately for the STR loci in each kit. Major and minor components were identified in each mixture sample (binary and ternary mix). All major component alleles were identified in both mixtures due to the small amount and the mixture of the three DNA samples, major alleles were detected at each locus of the three-person mixture sample. Minor alleles were also observed. However analysis of the results was performed by relying on the analysis settings determined in previous studies (Butler 2012). As a result, the ideal analysis was performed considering the average evaluation of all three kits with a stochastic threshold of 360 at an analytical threshold of 125 RFU and a DNA amount of 0.5-0.8 ng/µl. These values were considered as the average values containing STR loci of the three kits ("ENFSI Best Practice Manual December 2022 Human Forensic Biology and DNA Profiling," n.d.).

DISCUSSION

In forensic identification, crime scene and reference DNA samples are compared with the allelic ladder included in the kit with the help of the software. The formation of the analyzable peaks for each STR locus and the ratios of the peak levels observed among the heterozygous alleles provide information about the performance of the study. Optimizing these parameters is possible completing the ISO 17025, SWGDAM, and ENFSI requirements of each forensic biology laboratory. These studies

were carried out with known examples. For the next studies, optimization studies are planned with crime scene samples such as bones, teeth, and semen. Since the study was also conducted with reference DNA samples, the validation study was performed by comparing it with the data defined in the kit protocol. In the study, analysis of Investigator® 24Plex, GlobalFiler™, and VeriFiler™ Plus Kits was done by comparing the corresponding STR locus alleles in the allelic ladder of each kit. In the evaluations, the minimum criteria determined by the SWGDAM and ENFSI DNA working groups were taken into account. When the STR loci contained in each kit were evaluated in terms of sensitivity and reproducibility, it was possible to compare the STR loci by obtaining the full profile in samples with DNA amount of 0.5-0.8 ng/µl and control samples. DNA quality in biological samples obtained from the crime scene may be low and the amount may be very small. That's why the target is to obtain full profile DNA with the lowest amount of DNA. The STR loci GlobalFiler™, VeriFiler™, and Investigator® 24Plex were almost identical to the STR loci in all three kits, with minor differences. If the D2S441, D10S1248, D18S51, FGA loci in the GlobalFiler™ kit did not work in the biological sample obtained from the crime scene and the DNA profile could not be obtained, the analysis was completed by studying the D2S441, D10S1248, D18S51, FGA loci in the VeriFiler™ or Investigator® 24Plex (Ludeman et al. 2018; Green et al. 2021; "Validation and Assessment of the Investigator® 24plex QS Kit for Forensic Casework Application: Comparison with the PowerPlex® Fusion System and GlobalFiler™ PCR Amplification Kits" 2021) Thus, by performing multiple PCR amplification in the same biological sample, analyzable results were obtained with full performance, using complementary kits.

An internal validation study was conducted for the GlobalFiler™, VeriFiler™ and Investigator®24Plex PCR Amplification Kits for use in forensic DNA identification studies. These three kits were optimized for laboratory condition with the 3130xl genetic analyzer. The large number of STR loci to be analyzed in the kits increases the reliability of the results in forensic analyses such as paternity tests and kinship tests. After this research, reference and control DNA studies were used to determine the conditions and methods under which a biological sample from the crime scene should be studied in the laboratory. In this study, it was determined that by providing optimal conditions of GlobalFiler™, VeriFiler™Plus and Investigation® 24Plex kits in the laboratory, reliable and accurate results can be obtained in the analysis of forensic events to be studied in the future (Taupin 2019; Toom, Wienroth, and M'charek 2022). These studies were considered the beginning of forensic DNA identification studies, and the goal was to be included in studies compatible with today's developing technologies.

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CONSENT TO PARTICIPATE

Laboratory staff voluntarily fingertip blood collected with a lancet, hair, and buccal swab samples to experiment. We thank them for their contribution.

CONSENT FOR PUBLICATION

All authors reviewed the results and approved the final version of the manuscript.

AUTHORS' CONTRIBUTIONS

Not applicable.

COMPETING INTERESTS

Not applicable.

REFERENCES

Alvarez-Cubero, Maria Jesus, Maria Saiz, Belén Martínez-García, Sara M. Sayalero, Carmen Entrala, Jose Antonio Lorente, and Luis Javier Martinez-Gonzalez. 2017. "Next Generation Sequencing: An Application in Forensic Sciences?" *Annals of Human Biology* 44 (7): 581–92. https://doi.org/10.1080/03014460.2017.1375155.

"Best Practice Manual for the Internal Validation of Probabilistic Software to Undertake DNA Mixture Interpretation 2012." n.d., no. 001.

Butler, John M. 2012. *Advanced Topics in Forensic DNA Typing:Methodology*. USA: Elsevier.

2015. *Advanced Topics in Forensic DNA Typing: Interpretation*. Academic Press. https://www.sciencedirect.com/book/9780124052130/advanced-topics-in-forensic-dna-typing-interpretation.

Butler, John M., Michael D. Coble, and Peter M. Vallone. 2007. "STRs vs. SNPs: Thoughts on the Future of Forensic DNA Testing." *Forensic Science, Medicine, and Pathology* 3 (3): 200–205. https://doi.org/10.1007/s12024-007-0018-1.

Correa, H. S. D., G. Brescia, V. Cortellini, N. Cerri, and A. Verzeletti. 2019. "Validation Study and Forensic Applications of Investigator 24plex QS Kit." *Forensic Science International: Genetics Supplement Series* 7 (1): 320–22. https://doi.org/10.1016/j.fsigss.2019.09.122.

Elwick K, Mayes C, and Hughes-Stamm,S. 2018. "Comparative Sensitivity and Inhibitor Tolerance of GlobalFiler® PCR Amplification and Investigator® 24plex QS Kits for Challenging Samples." *Legal Medicine* 32 (May):31–36. https://doi.org/10.1016/j.legalmed.2018.01.006.

"ENFSI Best Practice Manual December 2022 Human Forensic Biology and DNA Profiling." n.d.

"ENFSI-Validation-Guideline-04012024.Pdf." n.d. Accessed June 18, 2024. https://enfsi.eu/wp-content/uploads/2024/02/ENFSI-Validation-Guideline-04012024.pdf.

"Evaluation of the Investigator® 24plex GO! Kit and Associated Allele Frequency Data for Four South African Population Groups." 2024. *Forensic Science International: Reports* 9 (July):100357. https://doi.org/10.1016/j.fsir.2024.100357.

"EZ1 Advanced XL Robotic Platforms and DNA Intestigator Extraction Kit Validation Report.Pdf." n.d. Accessed July 10, 2024. https://dfs.dc.gov/sites/default/files/dc/sites/dfs/page_content/attachments/EZ1%20Advanced%20XL%20Robotic%20Platforms%20and%20DNA%20Intestigator%20Extraction%20Kit%20Validation%20Report.pdf.

Green, Robert, Jennifer L. Elliott, Wilma Norona, Francine Go, Vivian T. Nguyen, Jianye Ge, Marc L. Short, Julio J. Mulero, and Chang Zhong. 2021. "Developmental Validation of VeriFiler™ Plus PCR Amplification Kit: A 6-Dye Multiplex Assay Designed for Casework Samples." *Forensic Science International: Genetics* 53 (July):102494. https://doi.org/10.1016/j.fsigen.2021.102494.

Hares, Douglas R. 2012. "Expanding the CODIS Core Loci in the United States." *Forensic Science International: Genetics* 6 (1): e52–54. https://doi.org/10.1016/j.fsigen.2011.04.012.

2015. "Selection and Implementation of Expanded CODIS Core Loci in the United States." *Forensic Science International: Genetics* 17 (July):33–34. https://doi.org/10.1016/j.fsigen.2015.03.006.

ISO/IEC 17025:2017. n.d. "ISO/IEC17025:2017." ISO. Accessed June 20, 2024. https://www.iso.org/standard/66912.html.

Kreamer M, Prochnow A, Bussmann M, Scherer M, Peist R, and Steffen C. 2017. "Developmental Validation of QIAGEN Investigator® 24plex QS Kit and Investigator® 24plex GO! Kit: Two 6-Dye Multiplex Assays for the Extended CODIS Core Loci." *Forensic Science International: Genetics* 29 (July):9–20. https://doi.org/10.1016/j.fsigen.2017.03.012.

Ludeman, Matthew J, Chang Zhong, Julio J Mulero, Robert E Lagacé, Lori K Hennessy, Marc L Short, and Dennis Y Wang. 2018. "Developmental Validation of GlobalFiler™ PCR Amplification Kit: A 6-Dye Multiplex Assay Designed for Amplification of Casework Samples." *International Journal of Legal Medicine* 132 (6): 1555–73. https://doi.org/10.1007/s00414-018-1817-5.

Qu, Weifeng, Feng Wang, Dan Sun, Yi Liu, Xin Jin, Zheng Gong, Jinjie Liu, et al. 2024. "Internal Validation of the GA118-24B Genetic Analyzer, a Stable Capillary Electrophoresis System for Forensic DNA Identification." *International Journal of Legal Medicine* 138 (2): 361–73. https://doi.org/10.1007/s00414-023-03106-x.

SWGDAM Recommendations, "4344b0_813b241e8944497e99b9c45b163b76bd.Pdf." n.d.https://www.swgdam.org/_files/ugd/4344b0_813b241e8944497e99b9c45b163b76bd.pdf.

Tao, Ruiyang, Chong Chen, Xiang Sheng, Ruocheng Xia, Xiaochun Zhang, Jingyi Zhang, Zihao Yang, Suhua Zhang, and Chengtao Li. n.d. "Validation of the Investigator 24plex QS Kit: A 6-Dye Multiplex PCR Assay for Forensic Application in the Chinese Han Population." *Forensic Sciences Research* 7 (2): 172–80. https://doi.org/10.1080/20961790.2019.1665160.

Taupin, Jane Moira. 2019. *Interpreting Complex Forensic DNA Evidence*. Boca Raton: CRC Press. https://doi.org/10.4324/9781351023788.

"TFS-AssetsLSGmanuals4477604.Pdf." n.d. Accessed July 22, 2024. https://assets.thermofisher.com/TFS-Assets%2FLSG%2Fmanuals%2F4477604.pdf.

Toom, Victor, Matthias Wienroth, and Amade M'charek, eds. 2022. *Law, Practice and Politics of Forensic DNA Profiling: Forensic Genetics and Their Technolegal Worlds*. London: Routledge. https://doi.org/10.4324/9780429322358.

"Validation and Assessment of the Investigator® 24plex QS Kit for Forensic Casework Application: Comparison with the PowerPlex® Fusion System and GlobalFiler™ PCR Amplification Kits." 2021. *Legal Medicine* 52 (September):101902. https://doi.org/10.1016/j.legalmed.2021.101902.

Wang, Dennis Y., Siddhita Gopinath, Robert E. Lagacé, Wilma Norona, Lori K. Hennessy, Marc L. Short, and Julio J. Mulero. 2015. "Developmental Validation of the GlobalFiler ® Express PCR Amplification Kit: A 6-Dye Multiplex Assay for the Direct Amplification of Reference Samples." *Forensic Science International: Genetics* 19 (November):148–55. https://doi.org/10.1016/j.fsigen.2015.07.013.

Zhong, Chang, Siddhita Gopinath, Wilma Norona, Jianye Ge, Robert E. Lagacé, Dennis Y. Wang, Marc L. Short, and Julio J. Mulero. 2019. "Developmental Validation of the Huaxia™ Platinum PCR Amplification Kit: A 6-Dye Multiplex Direct Amplification Assay Designed for Chinese Reference Samples." *Forensic Science International: Genetics* 42 (September):190–97. https://doi.org/10.1016/j.fsigen.2019.07.001.

LIST OF ABBREVIATIONS:

CODIS: Combined DNA Index System

DNA: Deoxyribonucleic Acid

ENFSI: European Network of Forensic Science Institute

GCGA (JSGA): Gendarmerie and Coast Guard Academy

PCR: Polymerase Chain Reaction

RFU: Relative Fluorescence Units

STR: Short Tandem Repeats

SWGDAM: Scientific Working Group on DNA Analysis Methods