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# Validation of SureID® 21G Human STR Identification Kit Using Alternative Reaction Protocols

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## ABSTRACT

The SureID® 21G Human STR Identification kit, produced by Health Gene Technologies, is a next generation kit that amplifies 20 autosomal STR loci (including several CODIS and European Standard Set (ESS) loci) and the sex locus Amelogenin.The kit uses a fast- cycling PCR technology, which allows, in less than 2 hours, the amplification of 21 markers in a single multiplex assay using the 5-dye technology.

Main aim of the present study is to evaluate some critical parameters (sensitivity, reproducibility, precision, stochastic effects, intra/intercolor balance, peaks balance ratio, mixture detection) and the overall kit performance either on reference samples or on a wide variety of forensic samples when using reduced reaction volumes.

Furthermore, since forensic laboratories are looking for simple and rapid workflow to process single source samples and especially reference samples for DNA databases, we investigated the kit potentiality in case of direct amplification.

Results demonstrate that either at reduced reaction volumes than in case of direct PCR SureID® 21G kit is a reliable multiplex that allows rapid and efficient amplification of a wide variety of forensic samples, showing improved performance also on challenging casework samples as required by the forensic community for caseworks analysis as well as paternity testing.

## INTRODUCTION

During years, countries worldwide developed cDNA databases including profiles of certain categories of people and of biological evidence found at crime scene. This allows a routine comparison with a large number of DNA profiles, in order to increase the likelihood of identification as well as to link multiple crimes together.

The original CODIS Core Loci, required from October 1998 until December 31, 2016, included 13 loci. As of January 1, 2017, for uploading DNA profiles to the National DNA Index System, FBI required to expand CODIS loci with an additional 7 STR loci, in order to favorite international comparison with European databases that includes these loci [1-3]. Several commercial multiplexes have been developed to allow simultaneous amplification of a large number of these loci.

The SureID® 21G Human STR Identification kit™, produced by Health Gene Technologies, uses a fast-cycling PCR technology and the 5-dye system. It amplifies in less than 2 hours, in a single multiplex assay the sex locus Amelogenin plus the following 20 autosomal STR loci: D3S1358, TH01, D21S11, D18S51, Penta E,

D5S818, D13S317, D7S820, D16S539, CSF1PO, Penta D, vWA, D8S1179, TPOX, FGA, D19S433, D12S391, D6S1043, D2S1338 and D1S1656. In the present study we performed an internal validation of the SureID® 21G kit in order to evaluate some critical parameters (sensitivity, reproducibility, precision, stochastic effects, intra/intercolor balance, peaks balance ratio, mixture detection) and the overall kit performance either on reference samples than on a wide variety of forensic samples, when using reduced reaction volumes. Investigated parameters are the same. Finally we investigated the kit potentiality in case of direct amplification of a wide range of representatives forensic samples, due to the advantage of this procedure to saving money and time spent on processing DNA samples [4].

## MATERIALS AND METHODS

### DNA samples

Female DNA Control 9947A (2ng/µl) available in the SureID® 21G kit, male DNA Control 9948 (0.1ng/µl), male DNA Control 007 (0.1ng/µl), were used as "reference samples" for the experiments, while a wide range of casework samples previously analyzed in our laboratory were used as "real samples" (Table1). Details about caseworks samples type, number and preparation are described in par. 2.13.

Table 1: casework samples analyzed by regular protocol, reduced volumes and direct PCR.

| Sample Type | Standard PCR | Reduced PCR | Direct PCR | Sample Type | Standard PCR | Reduced PCR | Direct PCR |
|---|---|---|---|---|---|---|---|
| Liquid Blood | yes | yes | yes | Epithelial cells on clothes | yes | | yes |
| Blood on Copan paper | yes | yes | yes | Contact trace (googles) | yes | yes | yes |
| Blood on FTA paper | yes | | yes | Contact trace (glove) | yes | | yes |
| Bloodstain on denim (blue/black) | yes | yes | | Contact trace (keyboard) | yes | | yes |
| Blood on knife blade | yes | | | Contact trace (computer mouse) | yes | yes | |
| Blood on leather | yes | yes | | Fingerprint on a gun handle | yes | | yes |
| Blood with soil | yes | | | Cadaveric Tissue | yes | yes | |
| Cigarette | yes | yes | yes | Bone (1year) | yes | yes | |
| Liquid Saliva | yes | yes | yes | Carbonized Tissue | yes | yes | |
| Oral swab | yes | yes | yes | Tooth (Molar-1year) | yes | yes | |
| Chewingum | yes | | yes | Contact trace (scarf) | yes | | yes |
| Toothbrush | yes | yes | | Washed bloodstains on cotton | yes | | yes |
| Saliva on plastic bottle | yes | | yes | Fingernail | yes | | |
| Saliva on glass | yes | | yes | Paraffined Tissue | yes | yes | |
| Saliva on cotton | yes | | yes | FIngerprint on window | yes | | yes |
| Saliva on Copan paper | yes | | yes | Inked paper (contact trace) | yes | yes | |
| Saliva on FTA paper | yes | yes | yes | Saliva on Cigar | yes | | |
| Saliva with soil | yes | | | Contact trace Door handle | yes | | |
| Liquid Sperm (condom) | yes | yes | yes | Decomposed tissue | yes | yes | |
| Sperm on clothings | yes | | yes | Contact trace (scotch tape) | yes | | |
| Sperm with soil | yes | yes | | Epithelial cells on a balaclava | yes | yes | |
| Nasal mucus | yes | | | Print on a bullet | yes | | yes |
| Hair root anagen | yes | yes | yes | Washed bloodstain on denim | yes | yes | |
| Shaving razors | yes | | | Hair root telogen | yes | | yes |
| Fingernail scrapings | yes | yes | | Fingerprint developed with DFO | yes | yes | |

### PCR amplification

Reaction setup and thermal cycling were performed according using half of the volumes recommended in the manufacturer protocol. PCR reaction was prepared mixing 6.25µl of DNA PCR Master Mix and 3.12 µL SureID® 21G Primer Mix to which 2µl of DNA sample (0.5-1ng) and nuclease free H20 were added to obtain a final reaction volume of 12.5µl. Positive and negative controls (respectively Control DNA 9947A at 2ng/µl and DNase/RNase-Free water) were included. Amplification was performed in the thermal Cyder GeneAmp PCR System 9700 (aluminum block) using the 9600 emulation mode settings and the following PCR conditions: initial denaturation at 95°C for 5 min followed by 29 cycles of denaturation at 94°C for 10s, annealing at 61°C for 1min, extension at 70°C for 30s. The recommended quantity of DNA sample used is between 0.5ng and 4ng [5].

### Samples electrophoresis

Samples were prepared by combining 10ul of Hi-DI Formamide with 0.5µl of DNA Size Standard 500 Plus and

adding 9 µL mixture of reagents to 1 µL PCR product for each well of the 96 well plate. The Size-500 Plus contains the following 20 orange dye-labeled DNA fragments: 75, 87, 100, 117, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475 and 500 (bp). In addition, 1 µL of SureID® 21G Allelic Ladder Mix was added in one well of the sample plate. Plate was denatured at 95°C for 3 min and then cooled at 4°C for 3min [5].

PCR products were separated and detected on the multi-capillary sequencer ABI 3130 Genetic Analyzer (Life Technologies). Run conditions set up in the data Collection software were the following ones: Dye Set: SureID_21G (G5), Injection Time: 10s, Injection Voltage: 3kV, Electrophoresis Voltage: 15kV, Run Time: 1500 seconds in the POP4 polymer with a run temperature of 60°C.

Raw data were elaborated by GeneMapper® v.3.2 software with the following standard conditions: analytical threshold 150RFU (that was lowered to 50RFU in case of low DNA quantity), stutter peaks < 15%, Heterozygous Peak Height Ratios ³ 70% [5].

**Sensitivity study**

DNA quantity affects typing results: too much DNA can result in off-scale data and incomplete A-nucleotide addition, while extremely low quantity can produce unbalanced amplification. Serial two-fold dilutions of Control DNA 9947A (2ng/µl) were made to give final concentrations of 0.5 ng, 0.25ng, 0.125, 0.062, 0.031, 0.016 ng per reaction. DNA dilutions were tested in replicates using 28, 29 and 30 cycles and assessed for the number of alleles detected.

**Reproducibility study**

1ng of Control DNA 9947A (2ng/ul) and DNA Control 9948 (0.1ng/µl) were analyzed in separate amplifications during 4 subsequent days by 2 different operators in order to evaluate the reproducibility of genotype obtained. In order to eliminate variations in peak size and height data, the same conditions were used for capillary electrophoresis and analysis on ABI3130 instrument.

**Precision study**

Precision was evaluated by amplifying 1ng of Control DNA 9947A (2ng/µl) in quadruplicate and calculating size deviation (D.S.) for all 21 loci. In order to eliminate variations in peak size and height data, the same conditions were used for capillary electrophoresis and analysis on ABI3130 instrument.

**Stochastic effects**

Stochastic effects generally occur with the analysis of low quantity and/or low quality DNA samples and yield allele or locus drop-out. Serial dilutions of Control DNA 9947A (2ng/µl) were made to give final concentrations from 0.5ng to 0.01ng per reaction in order to evaluate the stochastic effect and to empirically determine an internal stochastic threshold (the peak height value above which it is reasonable to assume that, at a given locus, allelic dropout did not occur).

**Intracolor balance (ICB), intercolor balance and peaks balance ratio (PHR)**

Three different types of peak balance were evaluated to assess the overall kit balance. We collected 3 types of samples from 15 known donors volunteers (liquid blood,oral swabs, bloodstains on FTA card).

DNA was extracted from 10ul of liquid blood, an entire oral swab and a 5x5mm card punch by the Prepfiler Forensic DNA Extraction kit (Applied Biosystems) according to the Manufacturer Protocol. Samples were eluted in 50ul of Elution Buffer and then quantified in a 7300 Real Time System. By the Human Quantifiler kit (Applied Biosystems) according to the Manufacturer Protocol.The amplification of 1 ng of DNA was performed according to the SureID® 21G Human STR protocol using 29 cycles. Results were used to calculate peak height ratios for intracolor balance (ICB), intercolor balance and peaks balance ratio (PHR).

**Mixture study**

Forensic casework samples may contain DNA from more than one individual: therefore, it is essential to ensure that the typing system is able to detect all components in the DNA mixture. In order to reproduce the same experiment of the Manufacturer, mixtures of male Control DNA 9948 (0.1ng/µl) + female Control DNA 9947A (2ng/µl) were examined at various ratios (1:1, 1:5, 1:10, 1:15, 1:20). In addition, further mixtures in the same ratio were prepared using male Control DNA 007 (0.1ng/ul) + female Control DNA 9947A (2ng/µl).

The total amount of mixed genomic DNA was 1ng, at each ratio in both experiments. For the amplification 29 cycles were used and an analytical threshold of 50RFU was applied in order to detect the minor component.

**Casework samples study**

The ability to obtain results from DNA recovered from biological samples has been documented analyzing 50 different casework samples (Table1). DNA was extracted by the Prepfiler Forensic DNA Extraction kit (Life Technologies) Extraction kit according to the Manufacturer Protocol from the following samples: 10µl of biological fluids, entire oral swabs or swabs used to collect contact traces, 5x5mm stains on different substrates, 50mg soil mixed with biological fluids, 5x5mm tissues, 5 mm fingernail, 3 mm cutting from hair root. DNA was extracted by the Prepfiler BTA Forensic DNA Extraction kit (Life Technologies), according to the Manufacturer Protocol, from the following samples: 3×3×5-mm of chewingum, 5×5mm of scotch, 50 mg of pre-decalcified bones and teeth were extracted by Prepfiler BTA. All above samples were eluted in 50ul of Elution Buffer.

All samples were then quantified by the Human Quantifiler kit in a 7300 Real Time System (Life Technologies) and by the Investigator® Quantiplex HYres using the Rotor-Gene® Q (Qiagen). Positive and negative controls were used during extraction and quantification steps. All samples were amplified in duplicate using SureID® 21G Human STR and NGM Select™ (Applied Biosystems) according to manufactures protocols using 29 cycles for both kits [5-9]. In addition DNase/RNase-Free water was used as negative controls, while Control DNA 9947A (2ng/µl) for SureID® 21G and Control DNA 007 (0.1ng/µl) were tested as positive controls respectively for SureID® 21G and for NGM Select, during above amplifications. PCR products electrophoresis and data analysis were performed as described in Par.2.3.

**Direct amplification**

Laboratories are looking for simple, rapid and high throughput workflow to process single source samples and especially reference samples for DNA databases. Thus we further investigated the kit potentiality for direct amplification. 25 samples already amplified according to the regular protocol were selected and processed directly as representative of a wide range of forensic samples (Table 1).

A 1.2 mm punch taken from stains and swab sample, 1µl of liquid biological fluids (blood, saliva, semen), an entire hair root were added to 25µl of Master Mix. Positive and negative controls were also tested (Control DNA 9947A (2ng/µl) and DNase/RNase-Free water) according to manufacturer protocol and using 29 cycles.

## RESULTS AND DISCUSSION

The SureID® 21G Human STR Identification kit™ (Health Gene Technologies) uses a fast PCR fast-cycling PCR technology, which allows in less than 2 hours the amplification, in a single multiplex, of 21 markers (including several CODIS and ESS loci) using a 5-dye technology. To verify the kit reliability when using reduced reaction volumes, some critical parameters such as sensitivity, precision, reproducibility, stochastic effects, intra/intercolor balance, peaks balance ratio, mixture detection have been studied.

**Sensitivity study**

Full profiles were obtained reproducibly with 0.032ng of input DNA while occasional drop out was observed with DNA quantity less than 32pg due to stochastic effects (Par.3.7). Robust amplifications and full profiles were obtained with cycle numbers of 28, 29, and 30 using a threshold of 50RFU for allele calling, in order to detect also low DNA. However, 30 cycle amplifications, while increasing low DNA sample recovery, produce more artifacts (i.e. pull-up peaks). In our opinion 29 cycles give the best ratio quality/quantity when working with low DNA samples (Figure 1).

**Reproducibility study**

Allele assignments for Control DNA 9947A and Control DNA 9948 were identical during different analyses performed in 4 successive days by 2 different operators performed in the same electrophoresis and data analysis conditions. All profiles were correspondent to the controls known genotypes (Figure 2).

**Precision study**

The measurement error in sizing is determined by the degree of precision in sizing an allele multiple times. Precision is measured by calculating the standard deviation in the size values obtained for an allele that is run in several injections on the capillary sequencer.

By replicating Control DNA 9947A samples in the same electrophoresis and data analysis conditions, we found size deviation for all 21 loci included in the kit were within 0.15bp (Figure 3). Since sizing differences may occur between runs on the same instrument and between runs on different instruments,

hence each laboratory should evaluate D.S. to allow accurate and reliable genotyping.

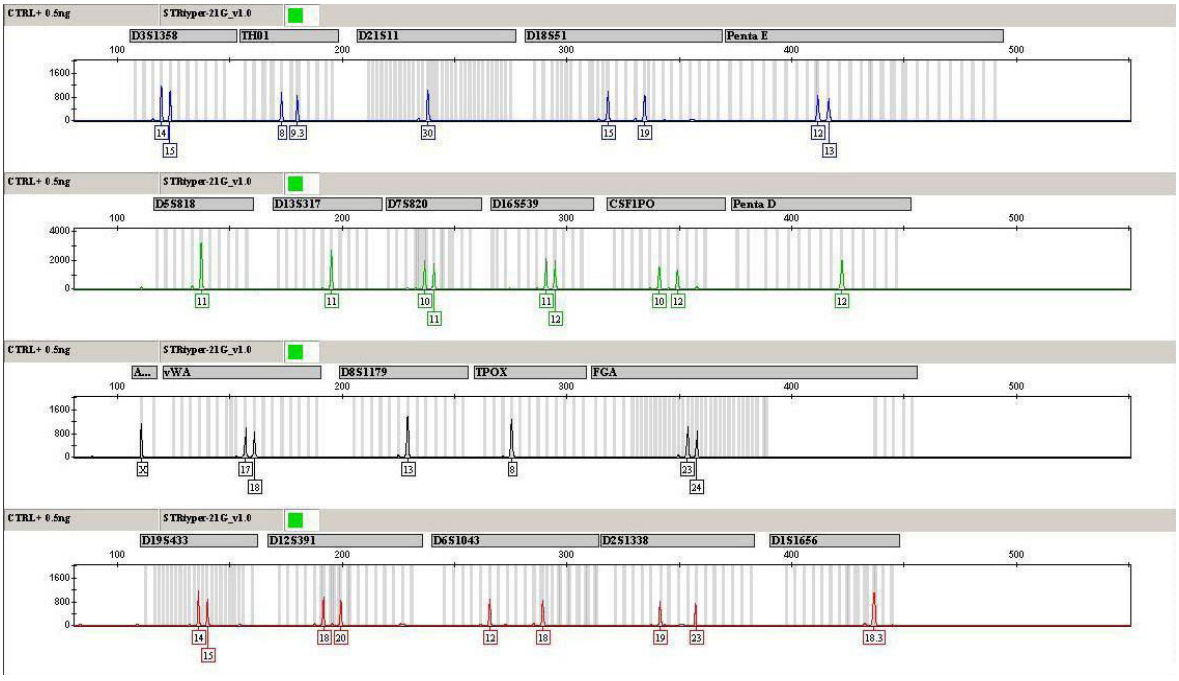


Figure 1: Full profile obtained from the amplification of 0.5 ng of Control DNA 9947A (Sensitivity Study).

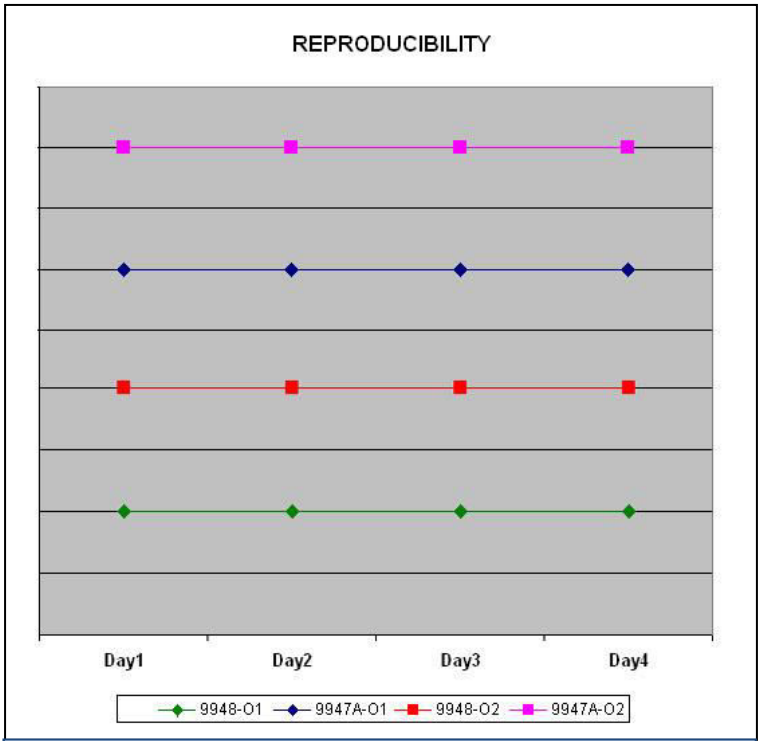


Figure 2: Concordance between data obtained by 2 operators, amplifying 1ng of Control DNA 9947A (2ng/ul) and DNA Control 9948 (0.1ng/⌈l) during 4 subsequent days (Reproducibility Study).

**Stochastic effects**

Serial dilution of Control DNA 9947A were used to study the stochastic effect. Full DNA profiles were observed using DNA quantity down to 32pg at a 50RFU (raw fluorescence unit) threshold for the analysis. Below that amount, allele and locus drop-out would occurred and partial DNA profile generally resulting from low quantity/quality samples. The sensitivity of the capillary electrophoresis instrument and the setting of the threshold of detection strongly influence the outcome.According to this study we established an internal stochastic threshold a value of 32pg of DNA at 50RFU (Figure 4).

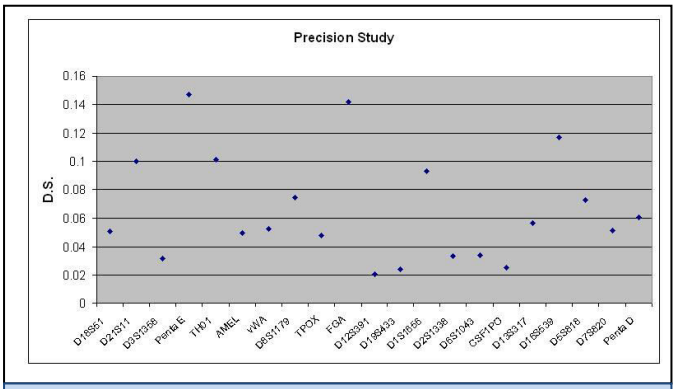


Figure 3: Size deviation (D.S.) values for all 21 loci included in SureID® 21G, calculated on data obtained by amplifying 1ng of Control DNA 9947A (2ng/ℓ) in quadruplicate. (Precision Study)

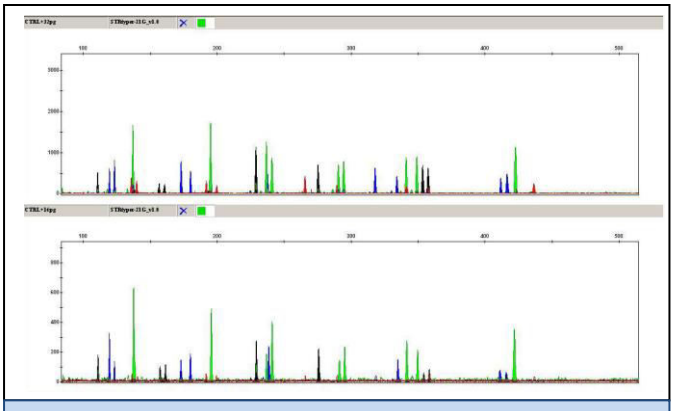

Figure 4: Full profile obtained from the amplification of Control DNA 9947A using 32pg and a partial profile with stochastic effects resulting using 16 pg of Control DNA 9947A.

**Intracolor balance (ICB), intercolor balance and peak height (PHR)**

Full profiles, with signals greater than 200 RFU obtained by amplifying 1ng of DNA from 45 samples were used to calculate intracolor balance, intercolor balance and heterozygote balance,using a Microsoft Excel sheet (Figure 5).

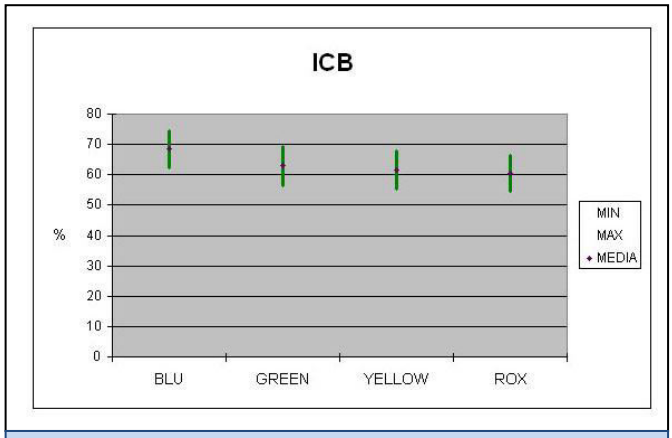


Figure 5: Mean, minimum and maximum intracolor balance (ICB) calculated as the ratio of the minimum average peak height within a dye channel to the maximum average peak height within the same dye channel.

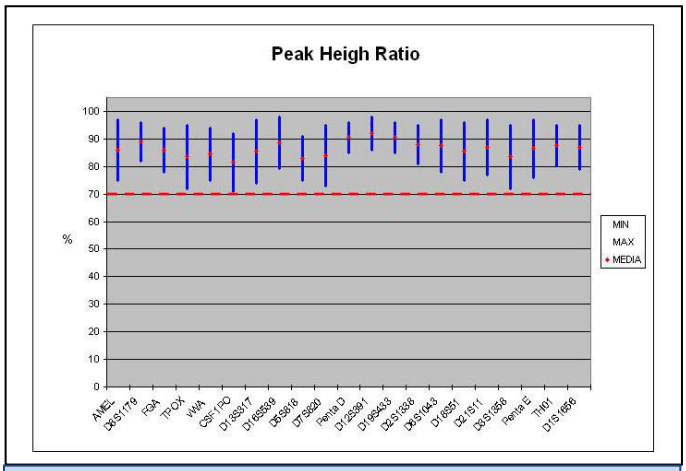


Figure 6: Mean, minimum and maximum peak height ratios (PHR) calculated for all 21 markers at heterozygote loci by comparing the RFU values of each allele and dividing the lower RFU value by the higher value.

The Intracolor Balance (ICB) was calculated as the ratio of the minimum average peak height within a dye channel to the maximum average peak height within the same dye channel. Intracolor balance, calculated within each group of loci sharing the same dye, yielded a value >40%.

Intercolor balance was calculated as the ratio of the minimum average peak height to the maximum average peak height regardless of dye color. Intercolor balance was estimated to be within a range of 23%-42%. A completely balanced profile would be indicated by all dye colors representing at least 20% of the total signal.

PHRs (Peak Height Ratio) were calculated for heterozygous genotypes for all markers by comparing the RFU values for each allele and dividing the lower RFU value by the higher

value. In all cases the ratio has to be multiplied by 100 to express value as percentage. Heterozygote ratios for 1 ng of input DNA were >70% (Figure 6). The minimum average peak height calculated was 71%.

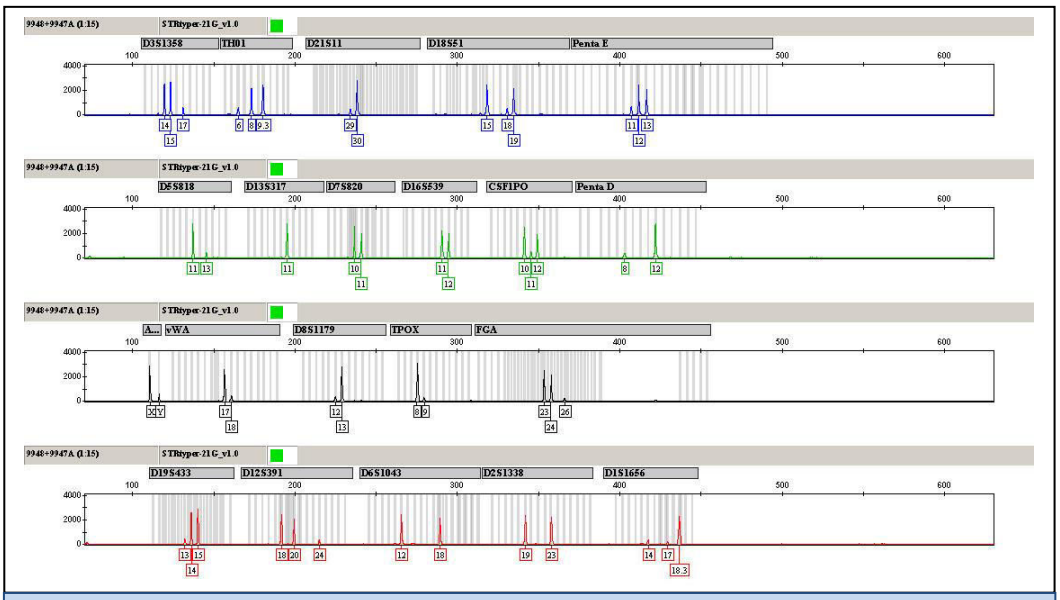


Figure 7: Full DNA profile of a mixture consisting in Control DNA 9948 (male) + Control DNA 9947A (female) in ratio 1:15 (1ng total DNA).

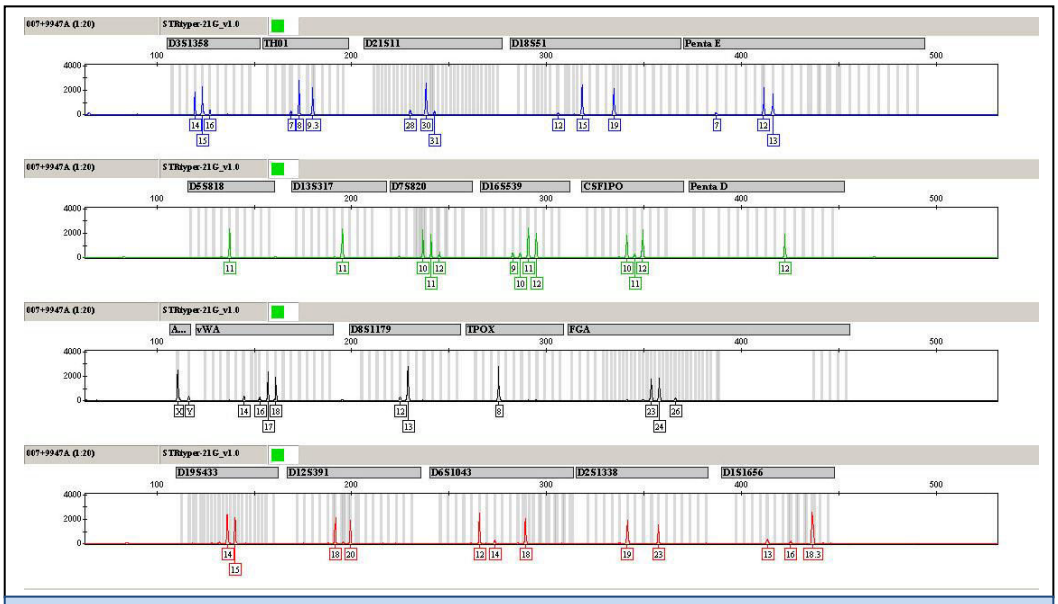


Figure 8: Partial DNA profile of a mixture consisting in Control DNA 007 (male) + Control DNA 9947A (female) in ratio 1:20 (1ng total DNA) – it's observed a drop out of minor component alleles for loci D7S820, D12S391,D2S1338,PentaD.

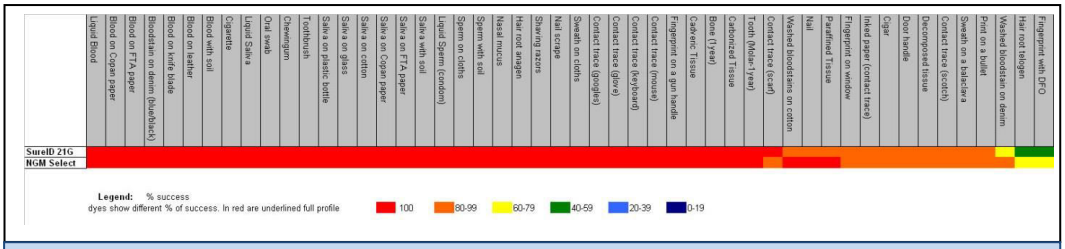


Figure 9: Heatmap showing a wide variety of casework samples profile completeness ordered, left to right, best to worst as comparison between SureID® 21G and NGM Select™.

**Mixture study**

Two different groups of mixtures were examined at various ratios (1:1, 1:5, 1:10, 1:15, 1:20): male Control DNA 9948 (0.1ng/µl) + female Control DNA 9947A (2ng/µl) and male Control DNA 007 (0.1ng/µl) + female Control DNA 9947A (2ng/µl).

Detection of full profiles for the minor contributor was possible down to ratio 1:15 (Figure 7), while 1:20 ratios resulted in partial profiles for the minor component (Figure 8). The limit of detection of the minor component was determined by analyzing non-overlapping alleles of both DNA samples. An analytical threshold 50RFU was used in order to detect as much as possible of the minor component.

**Casework samples study**

Genotyping performance of SureID® 21G and NGM Select™ has been compared in 50 casework challenging samples. The quality of STRs profiles obtained has been evaluated considering peaks balance, preferential amplification, allelic drop-out, etc.

Amplification was considered successful when were obtained reliable profiles with signals greater than 150 RFU (or 50 RFU for low DNA samples), an intralocus peak height ratio at each locus greater than 70%, no extra peaks or artifacts. Concordance between data obtained using other commercial forensic multiplex has been observed, even if different primers are used by different Manufacturers.

Profiles showed high quality and good peaks balance also from difficult samples: obviously different kind of samples at almost the same DNA concentration showed different typing success depending on the sample type. (Figure 9) It's in fact well known that the nature of the evidence, the substrate, the storing condition and the exposition to environmental factors (heat, humidity, UV, etc) have a big impact on DNA preservation and consequently on final results.

Due to the sensibility of the system, we observed an excess of DNA input results in artefact peaks and increased background noise, especially in green dye channel. Thus, we strongly recommend the use of correct DNA quantity (between 0.5 ng to 4 ng). Generally less DNA is preferable to too much DNA.

In addition partial profiles from difficult casework samples (degraded or low-template DNA) were informative enough, because almost half of the loci in the kit (D3S1358, TH01, D21S11, D5S818, D13S317, D7S820,D16S539, Amelogenin, vWA, D8S1179, D19S433, D12S391) produce amplicons less than 290bp.

Average rate of success for each locus has been calculated using an excel calculation sheet, validated internally in the lab. In the calculation formula per each locus has been included the number of times in which the marker gave a result on respect to the total number of assays performed. For example a value 100% indicates the locus was always successfully typed (Figure 10).

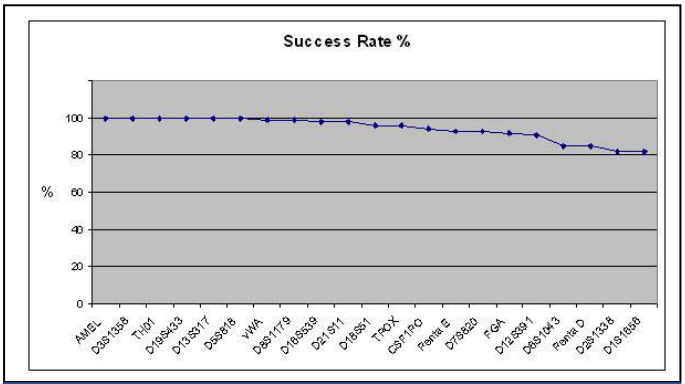


Figure 10: Average rate of success for each of the 21 loci included in SureID® 21G, determined by evaluating the completeness of DNA profiles obtained from casework samples.

**Direct amplification**

Almost all 25 analyzed samples (biological fluids, biological stains on different substrates, washed bloodstains, cigarette butts, prints, contact traces, hair roots) were successfully amplified (Figure 11) and gave good quality profiles when analyzed with a direct procedure in spite of the fact the kit was not originally developed for this application (Figure 12). Direct amplification performance has been determined evaluating the completeness of DNA profiles obtained from analyzed samples.

It has been used an excel calculation sheet, validated internally in the lab. In the calculation formula per sample has been included the number of loci which gave a results on respect to the total number of loci analyzed (21). For example a value 100% indicates a positive result for all 21 markers (full DNA profile).

The procedure is simple and rapid because it does not require any sample pre-treatment with a special buffer: an aliquot/fragment of the sample may be added directly to the amplification mixture taking caution to cover entirely the

sample with the PCR Master Mix. In case of liquid biological sample a pre-dilution (1:10) in DNAse/Rnase-Free water is suggested especially if working with fresh samples.

Due to the lack of purification step, direct amplification performs better with clear biological samples (saliva) or samples on FTA card or other clear substrates.

In addition the procedure is useful with low-template DNA samples (i.e. contact traces) because it reduces the risk of DNA loss during different steps of the extraction procedure.

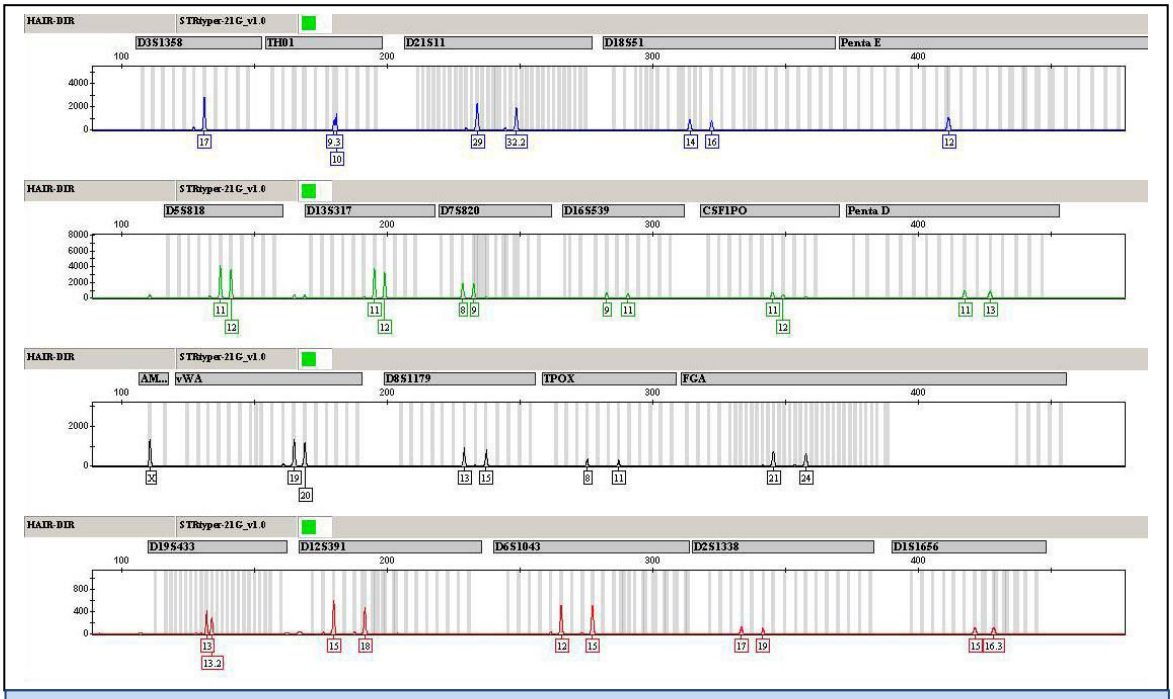


Figure 11: Example of a DNA profile obtained by direct amplification of a hair root (29 cycles).

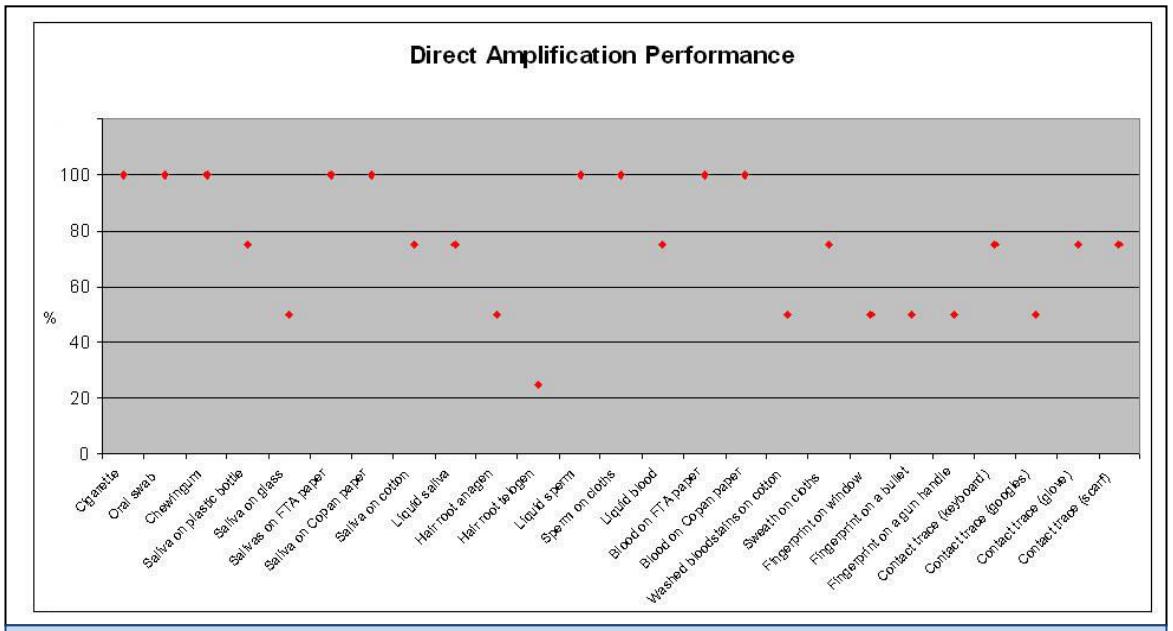


Figure 12: Direct amplification performance (expressed as percentage of rate success) of several representative casework samples.

## CONCLUSIONS

The present study was aimed to evaluate the SureID® 21G kit performance when using reduced amplification volumes or direct PCR in order to establish reliability and limitations in laboratory practices (SWGDAM, 2010). Our findings demonstrated that SureID® 21G kit is a reliable multiplex that allows rapid analysis of a wide variety of forensic samples. The system shows improved performance and sensitivity together with a great tolerance to high levels of PCR inhibitors; it allows maximum recovery of information even when working with challenging samples.

Profiles (28 cycles) were generally well balanced, with clear baseline, low noise and no PCR artefacts: 29 cycles give the best ratio quality/quantity when working with low DNA samples. Anyway, in case of very low or very high DNA amount we observed the presence of artefact peaks (in particular in green dye channel) and an increased background noise in comparison with the other 4 dyes channels.

Results showed the SureID® 21G kit may be successfully used also for the direct amplification of a wide range of forensic samples without the need for DNA extraction or purification. This minimizes costs, the risk of contamination and procedural errors by reducing the number of processing steps and it helps to accelerate time to result while maintaining good profile quality.

In conclusion SureID® 21G shows a robust PCR chemistry and the improved performance requested by the forensic community for challenging casework samples as well as paternity testing even when reaction protocols alternative to the Manufacturer one are used.

**Conflict of interest**

None

## REFERENCES


1. Gill P, Fereday L, Morling N, Schneider PM. (2006). The evolution of DNA databases recommendations for new European STR loci, Forensic Sci. Int. 156: 242-244.
2. Hares DR. (2015). Selection and implementation of expanded CODIS core loci in the United States, Forensic Sci. Int. Genet. 17: 33-34
3. Coble MD, Butler JM. (2005). Characterization of new miniSTR loci to aid analysis of degraded DNA, J. Forensic Sci. 50: 43-53.
4. (2004). Scientific Working Group on DNA Analysis Methods (SWGDAM), Revised Validation Guidelines, Forensic Sci. Communications.
5. (2015). User Guide-SureID® 21G Kits, HEALTH Gene Technologies.
6. Brevnov MG, Pawar HS, Mundt J, Calandro LM, Furtado MR, et al. (2009). Developmental validation of the PrepFiler Forensic DNA Extraction Kit for extraction of genomic DNA from biological samples. J For. Sci. 54: 599-607.
7. Green RL, Roinestad IC, Boland C, Hennessy LK. (2005). Developmental validation of the Quantifiler real-time PCR kits for the quantification of human nuclear DNA samples. Journal of Forensic Sciences. 50: 809-825.
8. Green RL, Lagacé RE, Oldroyd NJ, Hennessy LK, Mulero JJ. (2013). Developmental validation of the AmpFℓSTR® NGM SElect™ PCR Amplification Kit: A next-generation STR multiplex with the SE33 locus – FSI Genetics. 1: 41-51.
9. Butler J. (2012). Advanced Topics in Forensic DNA Typing, Elsevier.