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Typeability of DNA in Touch Traces Deposited on Paper and Optical Data Discs

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Background. Nucleated epithelial cells that are transferred by casual touching and handling of objects are the primary source of biological evidence that is found in high-volume crimes. Cellular material associated with touch traces usually contains low levels of DNA template making it challenging to acquire an informative profile.
Objectives. The main purpose of this study was to examine the efficacy of DNA typing in fingerprints deposited on optical data discs and the office paper.
Material and Methods. Latent fingerprints were made by 60 subjects of both sexes (30 males and 30 females). A highly effective DNA extraction method with QIAamp DNA Mini Kit (Qiagen) and an increased sensitivity PCR by AmpFlSTR® NGM™ Amplification Kit (Applied Biosystems) carried out at standard 30 cycles and at increased 34 cycles were used.
Results. The mean value of total DNA recovery was 0.4 ng from CDs/DVDs and 0.3 ng from the office paper. Amplification of Low Template DNA (LT-DNA) resulted in improved analytical success by increasing the number of PCR cycles from standard 30 to 34. On the other hand, the increased PCR cycles resulted in allele drop-ins showing additional peaks, the majority of which were outside the stutter positions.
Conclusions. Rigorous procedures and interpretation guidelines are required during LT-DNA for producing reliable and reproducible DNA profiles for forensic purposes (**Adv Clin Exp Med 2015, 24, 3, 437–440**).

Key words: forensic medicine, touch traces, genotyping techniques.

Since late 1990s the forensic community has focused on the analysis of DNA profiles related with volume crimes which cause the greatest impact on the society (such as burglary, shoplifting, vehicle crimes, street robbery and drug cases) [1–3]. The primary source of biological evidence that is found at high-volume crime scenes are nucleated epithelial cells that are transferred by casual touching and handling of objects, often referred to as "touch DNA". The amount of DNA that is recovered from touch DNA traces is usually below 100 pg (contained in approximately 15 diploid cells) that is typically optimized for standard multiplex PCR (Polymerase Chain Reaction). The likelihood of obtaining a full DNA profile from the surface of handled objects depends on the individual who has touched the object, which hand has been used, the activities of the handler prior to touching the object and the nature of the substrate [4]. Some of the scientific literature refers to the term LCN-DNA (Low Copy Number-DNA) to describe samples that contain low levels of DNA template where stochastic effects are present. LCN DNA was later accepted to define non-standard techniques used to improve the sensitivity of DNA profiling which include sample clean-up, increased PCR cycling and annealing time, and increased injection times [5–9], while Caddy et. al., introduced the term LT-DNA (Low Template-DNA) which refers specifically to samples that contain low levels of DNA [10]. The development of commercial autosomal Short Tandem Repeat (STR) kits has provided the possibility

of obtaining a DNA profile from multiple sources of biological material and contributed to a worldwide standardization and validation of this technology [11]. Previous studies have demonstrated that methods which need only small amplicon sizes to detect DNA markers were the most effective in the analysis of low template or degraded DNA. The primers for these markers were all designed to bind as close to the repeat region as possible to allow for the smallest possible amplicon, usually between 50 and 150 base pairs (bps) [12–14]. Compromised profiles are frequently subject to stochastic effects which complicate interpretation. They may include: heterozygote imbalance, missing alleles (allele drop-out), non-donor alleles due to minute amounts of contamination or slippage effects (allele drop-in) and increased stutter peaks [15, 16]. These complications have prompted a wide dispute on admissibility of LCN results interpretation in terms of legal proceedings [17]. The interpretation and limits of these effects are based on internal laboratory validation [18]. In case of low copy number DNA the research procedure developed by a laboratory shall be based on scientific publications and information specified by the manufacturers of technical equipment used for the research.

The objective of this research was to evaluate the efficacy of DNA typing in fingerprints deposited on optical data discs and the office paper using a highly effective DNA extraction method and an increased sensitivity PCR by AmpFlSTR® NGM™ Amplification Kit. While considering the types of carriers for touch traces in this research we have taken into account that data stored on CD/DVD discs may be of interest for law enforcement personnel in the context of incriminating evidence e.g. child pornography, sexual crimes, forgeries, white collar crimes; while anonymous letters may be related with extortion, kidnapping, or terrorist activities.

Material and Methods

The research was carried out on latent fingerprints made by 60 subjects of both genders (30 males and 30 females) who were asked to press and rub three fingers (thumb, index and middle finger) of both palms against recordable sides of optical data discs (CDs and DVDs) and sheets of office paper (jet 80 g/m^3). The volunteers did not wash their hands for a period of 2 h, performed standard office work and were asked to touch their hair and faces several times. Before the deposition of fingerprints the surfaces were UV irradiated in an Ultraviolet Crosslinker® (UVP) at 120 J/cm^2 for approx. 300 s in order to degrade any extraneous DNA. Sterile cotton swabs were used to collect trace material from the optical data discs. Traces from office paper were collected by cutting-out squares of 20 × 20 mm. The fingerprint samples were collected in triplicates. A total of 120 (60 + 60) samples were obtained. DNA extraction was performed with QIAamp DNA Mini Kit (Qiagen) using a modified method for epithelial cells. Quantifiler® Human DNA Quantification Kit and ABI 7500 Real Time PCR System (Applied Biosystems) were used for DNA quantitation according to the manufacturer's specifications. The limit of detection of this quantification kit is 0.023 ng/μL. Samples were amplified using the AmpFlSTR® NGM™ Amplification Kit in a GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems). The NGM Kit is a highly robust Short Tandem Repeat (STR) multiplex kit which utilizes a single protocol to amplify the ten AmpFlSTR®SGM Plus™ Amplification Kit loci (D2S1338, D3S1358, D8S1179, D16S539, D18S51, D19S433, D21S11, FGA, TH01, vWA, Amelogenin) five additional ENFSI/EDNAP recommended loci with two highly polymorphic STR loci (D1S1656 and D12S391) and three 'mini' STR loci (D10S1248, D22S1045 and D2S441). Two series of amplification reactions were carried out at standard 30 cycles and at increased 34 cycles in the presence of positive and negative controls. Final reaction volumes were adjusted proportionally to 10 mL. Reference profiles for each of the 30 donors were obtained using the standard cycling protocol using 500 pg DNA template as recommended by the AmpFlSTR® NGM™ Amplification Kit manufacturer. Results were compared to known donor profiles and drop-outs as well as drop-ins were noted. Genotyping of amplified DNA fragments was performed in 3130 Genetic Analyzer (Applied Biosystems) using a 10 s injection at 3 kV. Sample solution was 9 μL (174 μl HiDi formamide + 6 μL GeneScan™ 600 LIZ® Size Standard) and 1.2 μL DNA template. The data was collected using Data Collection v 3.0 software. GeneMapper ID-X v1.1.1 software was used for genotype classification (min peak height of 50 rfu (relative fluorescence units) for heterozygotes and 200 rfu for homozygotes). An allele was scored when its peak height was > 5% of the peak height of the most prominent allele at a given locus. False alleles were called when they were > 5% of the most prominent allele, but were not present in the genotype of the donor, as determined from a buccal swab control DNA sample. Partial profiles were assigned where at least 5 loci were typeable. Consensus profiles were generated for each sample where an allele is observed in common from 2 replicate reactions. Obtained results of experiments were used

as a basis to develop a research procedure in accordance with requirements of the EN ISO/IEC 17025:2005 norm.

Results

The amount of DNA recovered from a single surface was highly variable. The range of estimated total DNA recovery was 0–1.2 ng from CDs/DVDs (the mean value 0.4 ng) and 0–0.8 ng from the office paper (the mean value 0.3 ng). For each object type there was no significant difference between the amounts resulting from male and female volunteers. The typeability results obtained at 30 and 34 PCR cycles are summarised in Table 1. The extended amplification resulted in a profiling success increased by about 10%. The increase rates were similar for traces deposited on paper and optical data discs. The peak heights were also increased at 34 PCR cycles. For homozygous alleles, the peak heights ranged between 320 and 5450 rfu with an average height of 2460 rfu. The peak height range for alleles at heterozygous loci was 120 to 3800 rfu with an average height of 1660 rfu. The obtained results clearly indicate that increasing PCR cycles enabled typeability of samples containing less than 100 pg of DNA template. The alleles that were present and called showed signal strengths of 200 to 4200 rfu and were clearly distinguishable from baseline. Allele drop-outs were first observed the D2S1338, D18S51, D16S539 and FGA loci. In some of the null profile samples no alleles at any of the STR loci were present but the Amelogenin results were concordant with gender of the trace contributor. On the other hand, the increased PCR cycles to 34 resulted in allele drop-ins in 18 samples (15%) showing 52 additional peaks, the majority of which were outside the stutter positions.

Discussion

Interpretation of DNA profiles may become challenging when analyzing low template (< 100 pg/μL) or compromised samples in terms of allele dropout, band stutter and allele gains [7]. Commercial STR kits have been optimized and validated according to the FBI/National Standards and SWGDAM (The Scientific Working Group on DNA Analysis Methods) guidelines to produce good quality, balanced profiles with 1 ng of DNA with 28–30 PCR cycles. Our experiments represent an internal validation which has been accepted as complying with ISO/IEC 17025:2005 norm. During our studies, it has been shown that amplification of LT-DNA with AmpFlSTR® NGM™ resulted in improved analytical success by increasing the number of PCR cycles from standard 30 to 34. A further increase of the number of PCR cycles has been previously reported not to result in enhanced sensitivity [6]. Some authors question the improved sensitivity while indicating increased occurrence of undesirable artifacts due to stochastic effects which calls into question the reliability of the interpretation of results of LT-DNA analyses in current casework [7, 17]. On the other hand, using suitable protocols and interpretation guidelines in conjunction with an appropriate quality control program, a consensus approach has been approved for producing reliable and reproducible DNA profiles for forensic purposes, although developing aspects of evidence interpretation are still current [19].

Rigorous procedures required during LT-DNA analysis have been primarily aimed to avoid contamination by applying high quality chemicals, use protective clothing, separating individual stages of analysis, using negative controls and assessing of genotypes in cases of unequivocal results [6, 17, 20]. Increasing the number of PCR cycles would improve the sensitivity of the reaction compared with a standard cycle PCR. However, samples containing LT-DNA may not actually benefit from the increased amplification because of the additional alleles, either drop in or stutter, that can appear in the profile, which potentially would result in an incorrect genotype interpretation, and consequently false inclusion or exclusion of suspects, or false matches in DNA searches [21]. In our experimental conditions extra peaks were seen occasionally in full profiles. Misinterpretation of these results was compensated by setting the genotype classification criteria at min peak height of 50 rfu for heterozygotes and 200 rfu

Table 1. Detectability of DNA profiles from touch traced deposited on paper and optical data discs at 30 vs. 34 PCR cycles (slash separated)

Object	No. of samples	Full profiles	Partial profiles	Null profiles
CD/DVDs	60	14/16	34/37	12/7
Office paper	60	11/12	30/31	19/17
Total	120	25/28	64/68	31/24

for homozygotes. Non-specific peaks were possible to identify when they were > 5% of the most prominent allele, but were not present in the genotype of the donor. Obviously, this was possible, as reference profiles were known. Based on our experience, replicate analyses of sequential amplification products according to appropriate interpretation guidelines would also reduce miscalls in casework samples.

As demonstrated in this study and reported by other authors [22, 23] there was no significant difference between the amount of DNA deposited by male and female contributors.

In summary, when using AmpFlSTR® NGM™ we recommend increasing the number of PCR cycles from standard 30 to 34 to boost typeability of LT-DNA samples.

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