CESPU
INSTITUTO UNIVERSITÁRIO
DE CIÊNCIAS DA SAÚDE

Human Traces from Car Inner Surfaces

Broadening the Application of Genetic Policing

Sara Isabel Silva Moreira

Dissertation for the Degree of Master in Forensic Sciences and Laboratory Techniques

Gandra, May 2019

Human Traces from Car Inner Surfaces
Broadening the Application of Genetic Policing
Sara Moreira

IUCS - 2019

CESPU
COOPERATIVA DE ENSINO
SUPERIOR POLITÉCNICO
E UNIVERSITÁRIO

Sara Isabel Silva Moreira

Human Traces from Car Inner Surfaces
Broadening the Application of Genetic Policing

Dissertation for the Degree of Master of Forensic Sciences and Laboratory Techniques

Submitted to the University Institute of Health Sciences

Supervisor: Professora Doutora Áurea Marília Madureira e Carvalho, Instituto Universitário de Ciências da Saúde, CESPU, Gandra, Portugal; REQUIMTE/LAQV, Porto, Portugal; Faculdade de Farmácia da Universidade do Porto, Porto, Portugal.

Co-supervisor: Professora Doutora Maria Begoña Criado Alonso, Instituto Politécnico de Saúde Norte, CESPU, Gandra, Portugal; IINFACTS-CESPU, Gandra, Portugal.

Co-Supervisor: Dr. Pedro Nuno de Oliveira Correia, Serviço de Perícia Criminalística da Diretoria do Norte da Polícia Judiciária, Porto, Portugal.

Declaração de Integridade

Sara Isabel Silva Moreira, estudante do Mestrado em Ciências e Técnicas Laboratoriais Forenses do Instituto Universitário de Ciências da Saúde, declaro ter atuado com absoluta integridade na elaboração desta Dissertação.

Confirmo que em todo o trabalho conducente à sua elaboração não recorri a qualquer forma de falsificação de resultados ou à prática de plágio (ato pelo qual um indivíduo, mesmo por omissão, assume a autoria do trabalho intelectual pertencente a outrem, na sua totalidade ou em partes dele).

Mais declaro que todas as frases que retirei de trabalhos anteriores pertencentes a outros autores foram referenciadas ou redigidas com novas palavras, tendo neste caso colocado a citação da fonte bibliográfica.

Sara Isabel Silva Moreira

To my parents,

Isabel and António Jorge

Moreira, S., Lopes, M., Neto, A., Dinis-Oliveira, R., Correia, P., Alonso, M., Madureira-Carvalho, A. 2019. Extraction of DNA from buccal swabs: an optimization process. IV Congresso da Associação Portuguesa de Ciências Forenses/ XI Jornadas Científicas de Ciências do Instituto Universitário de Ciências da Saúde, Livro de resumos, Comunicação em poster nº14, p. 72-73.

Lopes, M., Moreira, S., Neto, A., Dinis-Oliveira, R., Correia, P., Alonso, M., Madureira-Carvalho, A. 2019. O ADN enquanto vestígio considerado prova em tribunal: casos reais de investigação criminal. IV Congresso da Associação Portuguesa de Ciências Forenses/ XI Jornadas Científicas de Ciências do Instituto Universitário de Ciências da Saúde, Livro de resumos, Comunicação em poster nº144, p. 231-232.

Moreira, S., Pereira, A., Dinis-Oliveira, R., Correia, P., Alonso, M., Madureira-Carvalho, Á. 2018. Forensic DNA and fingerprint databases. III Congresso da Associação Portuguesa de Ciências Forenses/ X Jornadas Científicas de Ciências do Instituto Universitário de Ciências da saúde, Livro de resumos, Comunicação em poster nº 30, p. 62-63.

Pereira, A., Moreira, S., Dinis-Oliveira, R., Correia, P., Alonso, M., Madureira-Carvalho, Á. 2018. Construção de uma base de dados lofoscópica. III Congresso da Associação Portuguesa de Ciências Forenses/ X Jornadas Científicas de Ciências do Instituto Universitário de Ciências da saúde, Livro de resumos, Comunicação em poster nº 31, p. 64-65.

AGRADECIMENTOS

"We seldom think of what we have, but always of what we lack."

Arthur Schopenhauer

Após este longo e exaustivo percurso, que envolveu inúmeros esforços pessoais e profissionais dada a exigência do tema escolhido e todas as dificuldades que foram surgindo, vejo agora concluída mais uma etapa académica da minha vida e, como tal, não podia deixar de agradecer a todos aqueles que me ajudaram a alcançá-la.

Começo por agradecer ao Professor Doutor Ricardo Dinis, não só pela excelência na docência, mas também pela admirável pessoa que é. Obrigada por todos os ensinamentos transmitidos e por toda a disponibilidade.

De todos os que contribuíram para a elaboração deste trabalho, devo um especial agradecimento à minha orientadora, Professora Doutora Áurea Carvalho, pelos conhecimentos transmitidos, pelo empenho, pela exigência, disponibilidade, paciência e motivação. Sou grata por nunca ter desistido de mim, nem nunca me ter permitido vacilar em momento algum. Desempenhou bem mais do que o seu papel e esteve comigo em todos os momentos!

Ao meu coorientador Dr. Pedro Correia, obrigada por toda a ajuda e transmissão de conhecimentos. Obrigada por ter aceitado a coorientação e com isso me ter permitido desenvolver parte do trabalho prático nas prestigiadas instalações da Polícia Jucidiária, fomentando assim os meus conhecimentos.

À minha coorientadora Professora Doutora Maria Begoña, obrigada por ter aceitado este desafio.

À Professora Doutora Filipa Carvalho, pela disponibilidade, dedicação e partilha de conhecimentos. Um especial agradecimento por nos ter "aberto as portas", tendo tal feito sido crucial para que o estudo não ficasse comprometido. À Dra. Ana Paula Neto, por toda a disponibilidade, ajuda, dedicação e empenho em todas as etapas conclusivas do trabalho de genética. Agradeço toda a preocupação manifestada, desejando e fazendo de tudo para que o trabalho corresse sempre pelo melhor.

Ao Especialista Adjunto da Polícia Judiciária, Victor Hugo Ferreira, por toda a amabilidade, disponibilidade, profissionalismo e conhecimento transmitido. Obrigada

mesmo! A sua dedicação e disponibilidade, desprovidas de qualquer "obrigação", foram inacreditáveis! Obrigada! Agradeço sinceramente também aos Especialistas Adjuntos João Paulo Faria e José Fonseca e ao Inspetor Rui Barreira, por toda a dedicação, compromisso e disponibilidade, todos foram excecionais! A todos os profissionais da Polícia Judiciária que tive a oportunidade de conhecer, pois sempre me receberam muitíssimo bem.

Ao Professor Doutor Daniel Mongiovi, muito obrigada, sem a sua contribuição este projeto não seria possível.

Ao investigador, produtor dos kits de genética, Andrew Lowftus, obrigada por toda a disponibilidade, acompanhamento e esclarecimentos prestados, tendo estes sido importantíssimos para a execução do estudo.

À Professora Doutora Juliana Faria e à Professora Joana Barbosa, muito obrigada por toda a disponibilidade e auxílio prestado no decorrer do trabalho prático de genética.

À Mariana Lopes, minha parceira de projeto, por toda a ajuda, esforço e pela partilha de bons momentos, mesmo quando o cansaço queria dominar.

À técnica Paula Vinhas que me ajudou muito com a logística dos materiais de laboratório necessários ao desenvolvimento do trabalho, tendo estado sempre disponível durante todos estes meses.

Aos meus pais e ao meu namorado, por todo o apoio, compreensão e esforço em todos os momentos difíceis e de cansaço extremo. Agradeço imenso a força constante e o facto de acreditarem em mim.

Às minhas estrelinhas, que estejam onde estiverem, sei que estarão sempre a torcer por mim.

Ao meu pequeno grande amor, que compreendeu quase sempre as minhas limitações de tempo e de disponibilidade.

Aos meus amigos que compreenderam a minha ausência durante esta fase e que sempre me motivaram e encorajaram.

Por fim, aos voluntários deste estudo, por toda a disponibilidade, empenho e recetibilidade em todas as etapas. O meu muito obrigada por o tornarem possível.

Projeto financiado pelo IINFACTS: programa de financiamento GID-CESPU de 2018, PI4AC: projeto HT-BAGP-CESPU-2018.

ACKOWLEDGEMENTS

"We seldom think of what we have, but always of what we lack."

Arthur Schopenhauer

After this long and exhaustive journey, which involved countless personal and professional efforts given the demand of the chosen theme and all the difficulties that have arisen, now I see another academic stage of my life completed and, as such, I could not fail to thank everyone that helped me to achieve it.

I begin by thanking to Professor Ricardo Dinis, not only for his excellence in teaching, but also for the admirable person he is. Thank you for all the teachings and for all the availability.

Of all those who contributed to the elaboration of this work, I owe a special thanks to my supervisor, Professor Áurea Carvalho, for the transmitted knowledge, the commitment, the requirement, availability, patience and motivation. I am grateful for never giving up on me, nor ever allowing myself to falter at any time. She did more than her role and was with me at all moments.

To my co-supervisor Dr. Pedro Correia, thank you for all the help and transmission of knowledge. Thank you for accepting the cosupervision, allowing me to develop part of my practical work in the prestigious facilities of the Polícia Judiciária (Scientific Police), fomenting my knowledge.

To my co-supervisor Professor Maria Begoña, thank you for accepting this challenge.

To Professor Filipa Carvalho, for the availability, dedication and shared knowledge. A special thank you for having "opened the doors" to us, what was crucial to do not have the study compromised. To Dra. Ana Paula Neto, for all the availability, help, dedication and commitment in all the conclusive stages of the genetic work. I thank for all the manifested concern, wishing and doing everything for a good development of the work.

To the Adjunct Specialist of the Polícia Judiciária Victor Hugo Ferreira, for all the kindness, availability, professionalism and transmitted knowledge. Thank you, a lot! His dedication and availability, devoid of any "obligation", were unbelievable! Thank you! I also sincerely thank to the Adjuncts Specialist João Paulo Faria and José Fonseca and the

Inspector Rui Barreira, for all the dedication, commitment and availability, all were exceptional! To all the professionals of the Polícia Judiciária that I had the opportunity to meet, since they always received me really well.

To Professor Daniel Mongiovi, thank you very much, without your contribution this project would not be possible.

To the researcher, who produced the genetic kits, Andrew Lowftus, thank you for all the availability, follow-up and clarification provided since they were very important to perform the study.

To Professor Juliana Faria and to Professor Joana Barbosa, thank you very much for all the availability and assistance during the practical work on genetics.

To Mariana Lopes, my project partner, for all the help, effort and sharing of good moments, even when tiredness wanted to dominate.

To the technician Paula Vinhas, that helped me a lot with the logistics of the laboratory materials that were needed to develop the work, being always available during all these months.

To my parents and to my boyfriend, for all the support, understanding and effort in all difficult moments and moments of extreme tiredness. Thank you so much for the constant strength believing in me.

To my little stars, wherever they are, I know that they will always cheer for me.

To my little love, that almost always understood my limitations of time and availability.

To my friends, who understood my absence during this period, always motivating and encouraging me.

Finally, to the volunteers of this study, for all the availability, commitment and receptivity in all stages. Thank you so much for making it possible.

Project financed by IINFACTS; 2018 financing program GID-CESPU; PI4AC: HT-BAGP-CESPU-2018 project.

RESUMO

A Lofoscopia e a Genética Forense são duas das Ciências Forenses mais importantes, contribuindo frequentemente para a resolução de uma enorme variedade de casos. Este conhecimento específico e direcionado é comumente necessário para analisar impressões digitais ou outros vestígios biológicos a partir dos quais é frequente a extração de DNA, visando sempre a identificação de um indivíduo. Reconhecendo o valor inegável dessas ciências, este trabalho consistiu na avaliação das dificuldades atuais em se conseguir identificações de indivíduos, analisando vestígios biológicos deixados em matrizes difíceis, analisando também a compatibilidade e a complementaridade dessas duas áreas de especialização. Assim, e uma vez que os crimes relacionados com furto e roubos de carros aumentaram consideravelmente em todo o mundo, doze voluntários, simularam a condução de um carro por 15 minutos, duas vezes. Após a condução, os volantes revestidos de pele, do primeiro ato de condução, foram analisados quanto ao DNA (método fenol-clorofórmio, InnoQuant HY-R® Kit, InnoTyper® 21 Kit) e, os volantes revestidos de pele, do segundo ato de condução, foram analisados quanto às marcas lofoscópicas (fumigação de cianoacrilato) e, sequencialmente, foram analisados quanto ao DNA. Previamente, foram construídas bases de dados lofoscópicas e genéticas para que fosse possível a identificação dos condutores de automóveis, por peritos de ambas as especialidades.

Apenas através da perícia genética, foi possível identificar dois terços dos condutores, enquanto que a realização da perícia lofoscópica em primeiro lugar, permitiu apenas identificar um terço. A mesma proporção foi obtida ao realizar-se a análise do DNA posteriormente à fumigação do cianoacrilato. Apesar de não inibir os reagentes dos kits, o cianoacrilato levou à redução da quantidade de DNA extraído, tendo-se obtido perfis genéticos com menor qualidade (menos marcadores amplificados), dificultando as possibilidades de obtenção de correspondências entre os perfis genéticos. Apenas metade dos condutores identificados através de perícia lofoscópica foram também identificados através da perícia genética, revelando que nem sempre existe uma correlação direta entre a eficácia de ambas as especialidades. Adicionalmente, o lado esquerdo do volante, tendo mais tempo de contato com a mão, permitiu também a obtenção de maiores quantidades de DNA, maior qualidade dos perfis genéticos e maiores taxas de correspondências entre

os mesmos. Além disso, o sexo masculino foi mais frequentemente identificado do que o sexo feminino.

Este trabalho, sugere que quando as forças policiais se depararem com este tipo de cenários, os esforços devem ser focados na execução da perícia genética, privilegiando-se o lado esquerdo do volante, no entanto são necessários mais estudos para confirmar os resultados aqui obtidos, os quais poderão contribuir para a definição de protocolos de ação ainda mais adequados para auxiliar o Sistema Judicial.

Palavras-chave: Identificação humana; Impressões digitais e palmares latentes; Cianoacrilato; Retrotransposões; InnoQuant® HY-R; InnoTyper® 21.

ABSTRACT

Lophoscopy and Forensic Genetics are two of the most important Forensic Sciences, frequently contributing to the resolution of a huge variety of forensic cases. This specific and targeted knowledge is commonly needed to analyse evidence like fingerprints or other biological traces from which it is frequent to perform DNA extraction, always aiming to achieve an individual identification. Recognizing the undeniable value of these sciences, this work consisted on an attempted to evaluate the current difficulties of achieving individual identifications by analysing biological traces left in challenging matrixes, also analysing the compatibility and complementarity of these both areas of expertise. For that, and since crimes related with car assaults and carjacking have been considerably increasing around the world, twelve volunteers, acting in a mock scene, drove a car for 15 minutes, twice. After driving, skin coated steering wheels, from the first act, were analysed for DNA (phenol-chloroform method, InnoQuant HY-R® Kit, InnoTyper® 21 Kit) and, skin coated steering wheels, from the second act, were analysed for lophoscopic marks (cyanoacrylate fuming) and sequentially for DNA. Lophoscopic and genetic databases were previously constructed to allow the identification of the car drivers through experts of both scientific arenas.

Performing solely genetic expertise, it was possible to identify two thirds of the car drivers, while performing lophoscopic expertise first, only allowed to identify one third. Same proportion was obtained when executing DNA analysis posteriorly to cyanoacrylate fuming. Despite not inhibiting the kits reagents, cyanoacrylate reduced the extracted DNA quantity, leading to the obtention of genetic profiles with less quality (fewer markers amplified), hampering the possibilities of achieving genetic matches. Only half of the car drivers identified through lophoscopic expertise were also identified through genetic expertise, revealing that there the is not always a direct correlation between the effectiveness of both expertise. Additionally, the left side of the steering wheel, having more time of hand contact, lead also to higher quantities of extracted DNA, higher quality of genetic profiles and higher rates of genetic matches. Moreover, males were more frequently identified than females.

This work suggests that when police forces met with this type of scenarios, efforts should be focused in the execution of genetic analysis, giving preference to the left side of the steering wheel. Further studies are needed to strengthen the results obtained herein and

contributing for the definition of even more suitable protocols of action to aid the Judicial System.

Keywords: Human identification; Latent palm and fingermarks; Cyanoacrylate; Retrotransposons; InnoQuant® HY-R; InnoTyper® 21.

INDEX OF CONTENTS

Index of Figures XXI

Index of Tables XXVII

Abbreviation List XXXI

I. Introduction 1

1.1. Forensic Sciences and biological traces 1

1.1.1 Human skin 1

1.2 Lophoscopy 3

1.2.1 Dactyloscopic type classification 4

1.2.2 Quiroscopic type classification 6

1.2.3 Minutiae classification 8

1.2.4 Development techniques 10

1.3 Forensic Genetics 12

1.3.1 Human identification and DNA analysis 13

1.3.1.1 Low copy number DNA - Touch DNA - Perspectives for new genetic markers 16

II. Aims 21

III. Materials and Methods 23

3.1. Volunteers recruitment and ethical issues 23

3.2. Ethical issues: volunteer´s privacy and codification 23

3.3. Creation of volunteer´s databases 24

3.3.1. Lophoscopic databases 24

3.3.1.1. Fingerprint database 24

3.3.1.2. Palmprint database 27

3.3.2. Genetic database 28

3.3.2.1 Collection of biological samples 29

3.3.2.2 DNA extraction 29

3.4. Mimetization of a real-life scenario driving a car 31
3.4.1. Car inner surface and make selection 31
3.4.2. Driving simulation and sampling destination 32
3.4.3. Laboratory practice avoiding steering wheels contamination 33
3.5. Forensic Expertise 33
3.5.1 Fingermark and palm mark analysis 33
3.5.2 Genetic analysis 36
3.5.2.1 Collection of evidence 36
3.5.2.2 DNA extraction 37
3.5.2.3 Assessment of DNA quantification and degradation 37
3.5.2.4 DNA typing 41
IV. Results and discussion 47
4.1 Volunteers data 47
4.2 Mimetization of a real-life scenario driving a car 49
4.3 Lophoscopy 50
4.3.1 Lophoscopic databases 50
4.3.1.1 Fingerprint database 50
4.3.1.2 Palmprint database 57
4.3.2 Lophoscopic expertise 60
4.3.2.1 Cyanoacrylate fuming 60
4.3.2.1 Hits and chartings 61
4.4 Forensic Genetics (database and expertise) 64
4.4.1 Assessment of DNA quantification and degradation 64
4.4.1.1 Running qPCR parameters evaluation 66
4.4.1.2 Sample DNA quantification 69
4.4.1.3 Sample Degradation Index 71

4.4.1.4 Male DNA Quantification 74
4.4.2 DNA typing 76
4.4.2.1 Multiplex PCR 78
4.4.2.2 Capillary eletrophoresis – fragment analysis 79
V. Conclusions 101
VI. References 103
VII. Attachments 109

Figure 1 - Skin stratification (adapted from (Ebling and Montagna, 2016b)). 2
Figure 2 - Dactyloscopic type classification (a) Adéltico; b) Monodéltico-Dextrodéltico; c) Monodéltico-Sinistrodéltico; d) Bidéltico/ Polidéltico) and dactylogram systems (1=Basilar system; 2=Marginal system; 3=Nuclear system). 5
Figure 3 - Right palmprint with a tripartite division (a=hypothenar; b=superior; c=thenar) 7
Figure 4 - Quiroscopic type classifications (a) Anucleado; b) Bucleado; c) Duplo bucle; d) Verticilo; e) Piniforme). 8
Figure 5 - Schematic representation of Transposable Elements. 17
Figure 6 – Ink pad for fingerprint collection (Sirchie). 25
Figure 7 - Magnifying glass to analyse fingerprints, palmprints and lophoscopic evidence. 26
Figure 8 – Set of materials for palmprint collection. 27
Figure 9 - Laboratory work under laminar flow chamber conditions. 28
Figure 10 - Collection of biological samples for constructing DNA database: a) harvesting cells from the buccal mucosa; b) sterile cotton swab. 29
Figure 11 – Statistical analysis of the European most stolen cars, in 2017 (adapted from (StolenCars24, 2017)). 31
Figure 12 – Driving simulation. 32
Figure 13 - Cleaning of a steering wheel after the application of the lophoscopic development reagent. 33
Figure 14 - Police evidence paper bag. 34
Figure 15 - Cyanoacrylate Projectina® fuming chamber: a) external view and b) internal view (blue arrow=aluminium container). 35
Figure 16 - Steering wheel being swabbed for collection of evidence after cyanoacrylate fuming. 36
Figure 17 - Real-Time Polymerase Chain Reaction (qPCR) Detection System (Bio-Rad CFX96 - C1000™ Thermal Cycler). 38
Figure 18 - InnoQuant® HY-R Human and Male DNA Quantification & Degradation Assessment Kit (InnoGenomics Technologies; right image missing the primer mix tube). 38
Figure 19 - Cycling Parameters for the qPCR of the InnoQuant® HY-R Kit. 40
Figure 20 - Applied Biosystems 3500 Genetic Analyser. 41

Figure 21 - InnoTyper® 21 Human DNA Analysis Kit (InnoGenomics Technologies). 41
Figure 22 - Multi-capillary IGT 5 Dye Matrix Standard (InnoGenomics Technologies). 42
Figure 23 - Cycling Parameters for multiplex PCR of the InnoTyper® 21 Human DNA Analysis Kit. 44
Figure 24 - Audi second hand steering wheels newly coated with skin. 49
Figure 25 - Metal structure constructed to handle Audi steering wheels. 50
Figure 26 - Dactyloscopic bulletin of the volunteer K (front part). 51
Figure 27 - Dactyloscopic bulletin of the volunteer K (reverse part). 51
Figure 28 - Quiroscopic bulletin of the volunteer K (front part). 57
Figure 29 - Quiroscopic bulletin of the volunteer K (reverse part). 58
Figure 30 - Driven steering wheels developed with cyanoacrylate fuming (A-L=volunteers codified according to the sampling sequence). 61
Figure 31 - Established "Hit" between the questioned fingerprint sample F (a)) and the reference fingerprint sample K (b)). 62
Figure 32 - Graphical demonstration of the established "Hit" between the questioned fingermark sample F (a)) and the reference fingerprint sample K (ring finger of the right hand; (b)). 62
Figure 33 - Graphical demonstration of the established "Hit" between the questioned palm mark sample H (a)) and the reference palmprint sample C (superior region of the right hand (b)) 62
Figure 34 – Graphical demonstration of the established "Hit" between the reference fingerprint sample L (a) and the questioned fingermark sample K (middle finger of the left hand (b)). 63
Figure 35 - Graphical demonstration of the established "Hit" between the questioned palmmark sample L (a)) and the reference palm print sample J (superior region of the right hand (b)). 63
Figure 36 - Alu and SVA retrotransposons illustration (InnoQuant® HY-R Human and Male DNA Quantification & Degradation Assessment Kit (adapted from (Pineda et al., 2014)). . 65
Figure 37 - Plate setup of the Real-Time Polymerase Chain Reaction of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing" (Std=standard; NTC=non template control; Unk=questioned samples; FAM HEX ROX, Cy5=amplification channels (fluorochromes)). 66

Figure 38 - DNA targets and respective amplification channels (fluorochromes; InnoQuant® HY-R Kit). 66
Figure 39 - Standard curves of the Real-Time Polymerase Chain Reaction of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing" (InnoQuant® HY-R Kit 67
Figure 40 - Internal Positive Control amplification of the Real-Time Polymerase Chain Reaction of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing" (same colours meaning same volunteers; InnoQuant® HY-R Kit). 68
Figure 41 - Short target amplification of the Real-Time Polymerase Chain Reaction of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing" (same colours meaning same volunteers; InnoQuant® HY-R Kit) 70
Figure 42 - Long target amplification of the Real-Time Polymerase Chain Reaction of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing" (same colours meaning same volunteers; InnoQuant® HY-R Kit) 72
Figure 43 - Sample Degradation Index of the Real-Time Polymerase Chain Reaction of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing" (N/A=not available; same colours meaning same volunteers; InnoQuant® HY-R Kit). 73
Figure 44 - Y Target amplification of the Real-Time Polymerase Chain Reaction of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing" (same colours meaning same volunteers; InnoQuant® HY-R Kit) 75
Figure 45 - Primer design strategy of *AluJ* markers of the InnoTyper® 21 Human DNA Analysis Kit (TSD=target site duplications; InnoGenomics Technologies (adapted from (Brown et al., 2017) 77
Figure 46 - Internal Lane Standard (IGT ILS-155) of the InnoTyper® 21 Human DNA Analysis Kit (addapted from (InnoGenomics Technologies, 2017)). 80
Figure 47 - Allelic Ladder information of the InnoTyper® 21 Human DNA Analysis Kit (addapted from (InnoGenomics Technologies, 2017)). 81
Figure 48 - Allelic Ladder information (markers, alleles and peak sizes) from the capillary electrophoresis of the research project "Human Traces from Car Inner Surfaces –

Broadening the Application of Genetic Policing" (InnoTyper® 21 Human DNA Analysis Kit). 82
Figure 49 - Negative control from the capillary electrophoresis of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing" (InnoTyper® 21 Human DNA Analysis Kit). 83
Figure 50 - Genetic profile of the Positive control from the capillary electrophoresis of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing" (InnoTyper® 21 Human DNA Analysis Kit). 84
Figure 51 - Genetic profile of the Reference Sample *Ja* from the capillary electrophoresis of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing" (InnoTyper® 21 Human DNA Analysis Kit). 85
Figure 52 - Number of markers without amplification in the genetic profile of questioned samples without cyanoacrylate from the capillary electrophoresis of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing" (InnoTyper® 21 Human DNA Analysis Kit). 86
Figure 53 - Number of markers without amplification in the genetic profile of questioned samples developed with cyanoacrylate from the capillary electrophoresis of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing" (InnoTyper® 21 Human DNA Analysis Kit. 87
Figure 54 - Matching rates of the profiles (complete and partial) from the questioned samples without cyanoacrylate of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing". 92
Figure 55 – Frequency of complete profiles, in samples without cyanoacrylate, according to the side of steering wheel. 93
Figure 56 – Percentage of complete profiles, in samples without cyanoacrylate, in both sexes. 93
Figure 57 - Matching rates of the profiles (complete and partial) from the questioned samples developed with cyanoacrylate of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing". 96
Figure 58 - Percentage of complete profiles, in samples developed with cyanoacrylate, according to the side of steering wheel. 97

Figure 59 – Percentage of complete profiles, in samples without cyanoacrylate, in both sexes. 98

Table 1 - Minutiae classification (types mainly considered in Portugal). 10
Table 2 - Principal techniques that are used to develop latent skin ridge marks, their mechanism of action and the nature of the surface where they should be applied. 12
Table 3 - Reaction Mix components and respective quantities per sample (InnoQuant® HY-R Kit). 39
Table 4 - Settings for preparing serial dilutions of the InnoQuant® DNA HY-R DNA Standard (InnoQuant® HY-R Kit). 40
Table 5 - PCR multiplex Reaction Mix components and respective quantities per sample (InnoTyper® 21 Human DNA Analysis Kit). 44
Table 6 - Capillary electrophoresis Reaction Mix components and respective quantities per sample (InnoTyper® 21 Human DNA Analysis Kit). 45
Table 7 – Characterization of the volunteers of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing". 47
Table 8 - Dactyloscopic types of the five fingers of the right hand and the five fingers of the left hand of the 12 volunteers (Olóriz System of Classification; *1 volunteer does not have the right forefinger; Bold=higher percentages). 52
Table 9 - Dactyloscopic types of the five fingers of the right hand and the five fingers of the left hand of the 6 female volunteers (Olóriz System of Classification; *1 volunteer does not have the right forefinger; Bold=higher percentages). 52
Table 10 - Dactyloscopic types of the five fingers of the right hand and the five fingers of the left hand of the 6 male volunteers (Olóriz System of Classification; Bold=higher percentages). 53
Table 11 - Mean values of the fingerprint minutiae of the five fingers of the right hand and the five fingers of the left hand of the 12 volunteers (*1 volunteer does not have the right forefinger; Bold=higher frequency; _=lower frequency). 54
Table 12 - Mean values of the fingerprint minutiae of the five fingers of the right hand and the five fingers of the left hand of the 6 female volunteers (*1 volunteer does not have the right forefinger; Bold=higher frequency; _=lower frequency). 55
Table 13 - Mean values of the fingerprint minutiae of the five fingers of the right hand and the five fingers of the left hand of the 6 male volunteers (Bold=higher frequency; _=lower frequency). 56

Table 14 - Quiroscopic types of the three regions of the right hand and the three regions of the left hand of the 12 volunteers (Olóriz System of Classification; Bold=higher percentages; -=not present; N/A=not applicable).....59
Table 15 - Quiroscopic types of the three regions of the right hand and the three regions of the left hand of the 6 female volunteers (Olóriz System of Classification; Bold=higher percentages; -=not present; N/A=not applicable). 59
Table 16 - Quiroscopic types of the three regions of the and and the three regions of the left hand of the 6 male volunteers (Olóriz System of Classification; Bold=higher percentages; -=not present; N/A=not applicable).....60
Table 17 - Information of the DNA markers of the InnoTyper® 21 Human DNA Analysis Kit (amplicon sizes measured in base pairs).....77
Table 18 - Amount of DNA input for Multiplex Polymerase Chain Reaction of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing" (ng=nanograms; yellow samples=partial expected profiles; green samples=none expected profiles; blue samples=complete expected profiles) 79
Table 19 - Genotypes of the reference samples from the capillary electrophoresis of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing" (I=insertion allele; N=null allele; ADO=allele dropout; *=Insertion allele with higher peak around 92bp, coincident with the marker artefact; +=Null allele with higher peak around 89bp, coincident with the marker artefact; InnoTyper® 21 Human DNA Analysis Kit). 89
Table 20 – Genotypes of the questioned samples without cyanoacrylate from the capillary electrophoresis of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing" (I=insertion allele; N=null allele; ADO=allele dropout; -=locus drop out; *=Insertion allele with higher peak around 92bp, coincident with the marker artefact; +=Null allele with higher peak around 89bp, coincident with the marker artefact; ►=peak interpretation doubt; InnoTyper® 21 Human DNA Analysis Kit).....90
Table 21 - Correspondence between questioned samples without cyanoacrylate and respective reference samples of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing" (LDO=locus drop out).....91
Table 22 - Genotypes of the questioned samples developed with cyanoacrylate from the capillary electrophoresis of the research project "Human Traces from Car Inner Surfaces –

Broadening the Application of Genetic Policing" (*=Insertion allele with higher peak around 92bp, coincident with the marker artefact; +=Null allele with higher peak around 89bp, coincident with the marker artefact; InnoTyper® 21 Human DNA Analysis Kit). 95
Table 23 - Correspondence between questioned samples developed with cyanoacrylate and respective reference samples of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing" (LDO=locus drop out)..... 96
Table 24 - Percentage of amplification efficiency of each marker of the InnoTyper® 21 kit when used in the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing" (*=marker with artefact peak). 99

ADO - Allele Drop Out

AFIS – Automated Fingerprint Identification System

cDNA – Complementary DNA

DI – Degradation Index

DNA – Deoxyribonucleic Acid

DTT- Dithiothreitol

EDTA-Ethylenediaminetetraacetic acid

INNUL – Insertion/ Null

IPC – Internal Positive Control

L1 – Long Interspersed Element-1

LCN – Low Copy Number

LDO – Locus Drop Out

LINEs – Long Interspersed Nuclear Elements

LPC-PJ – Laboratório Polícia Científica da Polícia Judiciária

LTRs – Long Terminal Repeats

mtDNA –Mitochondrial DNA

NTC – Non-template Control

bp – Base Pair

PCR – Polymerase Chain Reaction

PI – Principal Investigator

PJ – Polícia Judiciária

qPCR – Real-time Polymerase Chain Reaction

RFLP – Restriction Fragment Length Polymorphism

RM – Reaction Mix

RNA – Ribonucleic acid

SDS- Sodium dodecyl sulphate

SINEs – Short Interspersed Nuclear Elements

SNPs – Single Nucleotide Polymorphisms

SPR – Small Particle Reagents

Std. – Standard

STRs – Short Tandem Repeats

SVA – SINE-R/VNTR/Alu

TSDs – Target Site Duplications

TE – Transposable Elements

UV – Ultraviolet

VNTRs – Variable Number of Tandem Repeats

I. INTRODUCTION

1.1. FORENSIC SCIENCES AND BIOLOGICAL TRACES

In the last decades, Forensic Sciences became an extremely important tool to assist the application of justice, in the best interest of the citizen. Forensic Sciences can be considered as a multidisciplinary branch, which promotes the interaction of different scientific areas that put in practice their principles, practices and procedures, in order to help in the resolution of real cases related to the judicial or judiciary system (Saferstein, 1998). In most of the forensic cases (mainly related to criminal and civil law), there is a need to perform unequivocal identification of individuals (victims and/ or perpetrators) involved in illicit acts. This human identification can be achieved by analysing different biological matrixes obtained directly from the human body or presented as evidence at a crime scene, on different surfaces (Pinheiro, 2008). Biological evidence can have their origin in different parts of the body, being the human skin an excellent and common source.

1.1.1 HUMAN SKIN

Human skin (Figure 1) is the organ responsible for protection against aggressions of external agents, also regulating the body temperature and having sensory receptors associated with touch. It is formed by two layers, the internal layer named dermis and the outer layer named epidermis. The internal and lower anatomical layer is also referred as "the true skin", being essentially composed by elastin and collagen, arterial and blood capillaries, hair follicles and glands (Ebling and Montagna, 2016a). Sebaceous and sweat glands are present at dermis, being the sebaceous glands responsible for the secretion of sebum (mixture of lipids) and the sweat glands responsible for sweat production which mainly consists of water and other constituents (*e.g.* sodium chloride, potassium, ammonia, urea, lactate, uric acid, creatine, creatinine, amino acids) (Maceo, 2011). The epidermis mainly consists of keratinocyte cells which contain keratin, a protein responsible for water impermeability. Epidermis also contains Langerhans cells representing the immune system detecting foreign bodies and

protecting against infections and also Merkel cells, being essential for touch sensation (Baroni et al., 2012).

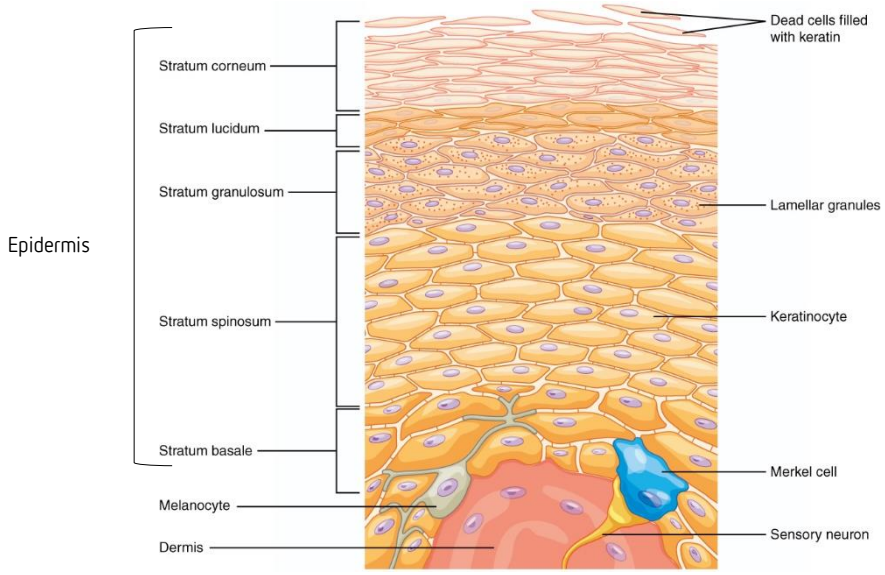


Figure 1 - Skin stratification (adapted from (Ebling and Montagna, 2016b)).

The epidermis is subdivided into five stratums. The deepest layer is the stratum basale consisting in cuboidal-shaped stem cells responsible for the origin of new keratinocytes trough mitosis, being the cells dragged to the upper strata. Consecutively, the stratum spinosum formed by polyhedral cells connected by desmosomes with increased keratin originating their grainy appearance; the stratum granulosum composed by more flattened cells with large amounts of keratin in the cytoplasm; the stratum lucidum constituted by keratinized cells who completed the process of cell death keeping the chemical activities until keratinization and finally, the outer layer, the stratum corneum mainly constituted by dead anucleated cells (keratinocytes highly differentiated) with high keratinization (Figure 1) (Maceo, 2011).

In the ventral part of the fingers, palms and feet, the epidermis outer layer presents ridges (small projections) interspaced by furrows that can be printed in a variety of surfaces due to sebum and sweat secretions covering the skin. At the same time that skin leaves its impression formed by sebum and sweat components, it can also leave some cells directly from the stratum corneum. The Locard Exchange Principle ("*Every contact leaves a trace*.") was clearly reflected when van Oorschot and Jones (van Oorschot and Jones, 1997) demonstrated that when handling objects, a small number of cells is transferred

(additionally to the transference of sebum and sweat) (Horswell, 2004). The secretions of the exogenous glands and the cell transference are influenced by several factors (individual and exogenous), leading to differences in the desquamation process and cell deposition between individuals, being the "shedder status" the donor's predisposition for cells deposition (Lagoa and Pinheiro, 2008; Skinauthority, 2019). Although humans release about 400 000 skin cells per day, the number of cells that are transferred by touching objects is variable (Singh Sankhla and Kumar, 2017). The influencing individual factors are mainly the age (affecting skin rejuvenation cycles), healthy condition (illness and stress), personal habits (*e.g.* nutrition) and perspiration, being the exogenous factors mainly related with the environmental conditions (*e.g.* weather temperature, humidity), type of receiving surface (*e.g.* rougher, porous) and time of contact (Correia, 2008). Latent skin impressions are mainly constituted by these biological components (sebum, sweat and cells), being commonly found as evidence in crime scenarios, on a huge variety of surfaces. Therefore, it is crucial the intervention of, at least, two forensic experts from two relevant forensic areas, Lophoscopy and Forensic Genetics, in order to fully characterize this type of evidence and achieve the identification of the donor.

1.2 LOPHOSCOPY

Lophoscopy is one of the oldest Forensic Sciences allowing the unequivocal identification of individuals, even differentiating monozygotic twins, by studying the dermopapillary skin ridge impression patterns (Patwari and Lee, 2008). Skin ridges form unique patterns and are used for human identification since they appear around the 4º/ 6º month of intrauterine life, being perennial until cadaveric decomposition (Principle of Perenniality) and not changing over time (Principle of Immutability), unless deep lesions occur in dermis. Additionally, they are different between different body parts of the same individual and also, and more important, between different individuals (Principle of Variability/ Diversity). Lophoscopy can be splitted in three subareas, depending on the skin pattern body provenance. Dactyloscopy relates to finger (distal phalange) skin impression patterns, while Quiroscopy is related to palm skin impression patterns and Pelmatoscopy is related to sole (foot) skin impression patterns (Auton et al., 2015; Correia, 2008). The epidermal ridge

patterns are generally called dactylograms, quirograms or pelmatograms, being classified as natural when directly observed in the finger, palm or sole, respectively. When located on surfaces, they can be classified as latent, being invisible evidence or classified as printed when they are visible and impregnated with ink, blood, or any other material. They can also be classified as moulded when the matrix (*e.g.* wax, cream) frames the impression (Peixoto and Ramos, 2010).

The latent dactylograms, also called latent fingermarks, are the ones that are more frequently found at crime scenes since they result from natural perspiration and since hands are usually naked, being the fingers used for almost everything. Once dactylograms are invisible, perpetrators have also difficulties in eliminating them, when trying. Palm marks are the second type of lophoscopic evidence that is mostly found and, lastly, footmarks that mainly appear at crime scenes related to sexual assault (Fawcett, 1970). Thus, knowledge related to fingerprints is well developed, being the knowledge about footprints scarce.

1.2.1 DACTYLOSCOPIC TYPE CLASSIFICATION

Fingerprints are widely used as a biometric parameter in order to identify individuals giving them benefits in different type of contexts. This biometric parameter can allow somebody, for example, to have access to restricted private areas or access to use a specific equipment (*e.g.* mobile phone). Even in the legal context, they are widely used and studied as evidence, not only due to their highly appearing frequency but also due to the low cost and high effectiveness associated with each forensic analysis (Correia, 2008).

In a dactylogram, epidermal ridges can be grouped in two or three systems, according to their organisation (shape and direction; Figure 2). The basilar system is present in all dactylograms and corresponds to the flat ridges immediately above the flexure fold (base of the distal phalange), being the upper flat ridge named basilar limitation. The marginal system is also present in all dactylograms and corresponds to the curved ridges on the top of the distal phalange, being the lower curved ridge named marginal limitation. The nuclear system does not exist in some dactylograms and, if present, corresponds to the central part of the pattern, being the upper nuclear ridge named nuclear limitation (Figure 2) (Correia, 2008).

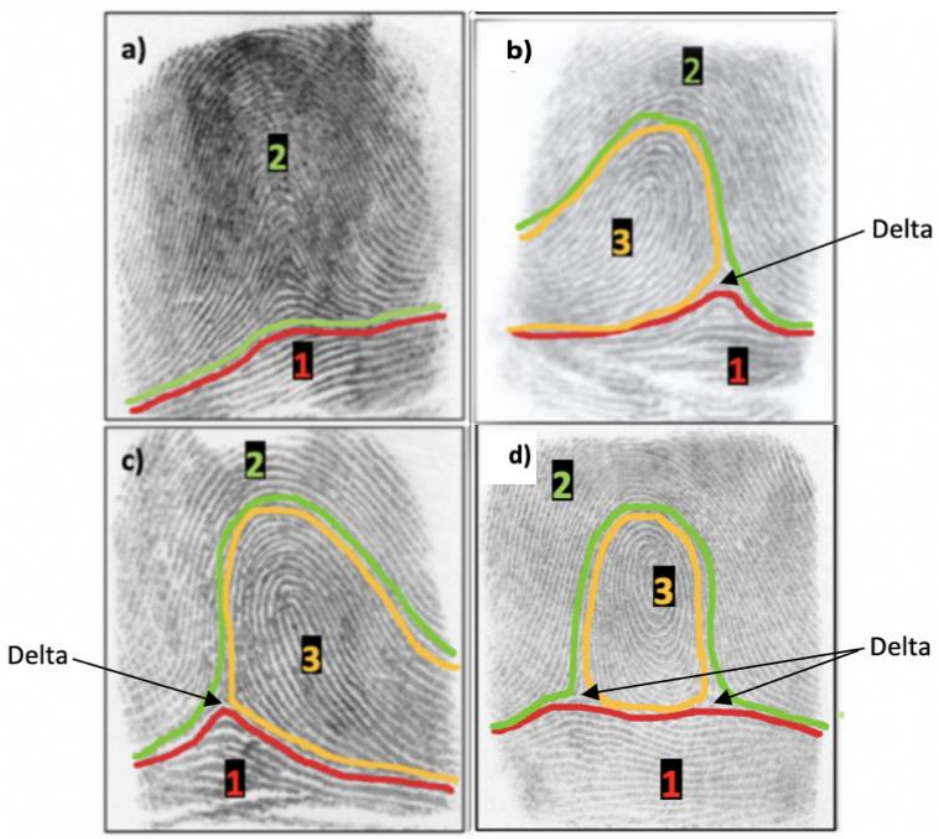


Figure 2 - Dactyloscopic type classification (a) Adéltico; b) Monodéltico-Dextrodéltico; c) Monodéltico-Sinistrodéltico; d) Bidéltico/ Polidéltico) and dactylogram systems (1=Basilar system; 2=Marginal system; 3=Nuclear system).

The observation of the delta figure (absence or presence; number and position) is crucial to classify the dactylogram pattern, also called dactyloscopic type. The delta presence is associated with the existence of the nuclear system, only appearing when all the limitations met at a specific area (Figure 2). The dactyloscopic type classification system used in Portugal is the Olóriz System of Classification, developed by Federico Olóriz Aguilera, being based on the previous Vucetich and Galton-Henry Systems (Vilar, 2015). The Olóriz System of Classification encompasses four main dactyloscopic types. The dactyloscopic type "Adéltico" only has the basilar and marginal systems, consecutively not having any delta figure (Figure 2.a). The dactyloscopic types "Monodéltico-Dextrodéltico" (Figure 2.b), "Monodéltico-Sinistrodéltico" (Figure 2.c) and "Bidéltico"/ "Polidéltico" (Figure 2.d) have the basilar, marginal and nuclear systems, having the figure delta in their pattern. "Monodéltico" type has only one delta, being its position very important to complete the

classification. If delta is on the right side of the fingerprint it is complementarily named as "Dextrodéltico" (Figure 2.b) and if delta is on the left side of the fingerprint it is complementarily called "Sinistrodéltico" (Figure 2.c). If two or more deltas are present, independently from their position, the dactyloscopic type is named as "Bidéltico" or "Polidéltico" (Figure 2.d) (Correia, 2008).

According to the Olóriz System of Classification, when collecting fingerprints from a victim or suspect, the dactyloscopic types should be codified, in order to construct a dactyloscopic formula that easily categorize the information. The dactyloscopic formula firstly presents the symbols of the right hand, starting by the thumb, and ending in the little finger of the left hand. Thumbs have the particularity of being identified using the first alphabetic letter of the specific dactyloscopic type, the remaining fingers are identified using Arabic numbers corresponding to the specific dactyloscopic types. Therefore, "Adéltico" type is encoded using an "A" or the number "1"; "Monodéltico-Dextrodéltico" type is encoded using a "D" or the number "2"; "Monodéltico-Sinistrodéltico" type is encoded using a "S" or the number "3" and "Bidéltico"/ "Polidéltico" type is encoded using a "V" or the number "4". The classification words "Bidéltico"/ "Polidéltico" starts with different letters, what leaded Olóriz to encode them using a "V", inspired in the correspondent type of the Vucetich Classification System, the "Verticilo" (Simas and Calisto, 2001). When fingers do not exist (*e.g.* amputation), the dactyloscopic type must be represented using the number "0" and if the fingertip has some anatomical problems (*e.g.* scar) making impossible the perception of the dactyloscopic type, it must be represented by the letter "X".

1.2.2 QUIROSCOPIC TYPE CLASSIFICATION

Palm mark traces also appear with some frequency in crime scenes and such as fingermarks they can also be classified and encoded. Palm marks are more frequently found in cylindrical objects (*e.g.* glasses, bottles, pipes) encompassing all the palm in order to be hold. However, due to the considerable palm area, these traces often appear incomplete/ fragmented, being the quirogram analysis more complex when compared with the analysis of dactylograms. Not rarely, understanding the orientation of the palm mark evidence discloses some difficulty (Simas and Calisto, 2001).

In Portugal, the classification of the quiroscopic types is also performed according to the Olóriz System of Classification, that considers the quirogram divided in three regions (Figure 3) which correspond to eminences of the hand palm: hypothenar (Figure 3.a), superior (Figure 3.b) and thenar (Figure 3.c) The hypothenar region is the most frequent in palm mark traces. Hypothenar ridges begin at the radial side of the superior region, gradually opening and covering almost of the entire hypothenar region (cubital eminence). Parallel to these ridges are two sets of ridges that begin at the basis of the indicator, opening to opposite hand sides. The separation between these three sets of ridges always promotes a formation of a delta in the superior region (Figure 3, green triangle). The superior region encompasses the interdigital eminences and has ridges that are parallel to the base of each finger, arching as they approach each flexure fold. The thenar region covers the thumb muscles area, having ridges parallel to the thumb on the radial eminence. Most of the palm traces that are usually found correspond to one of these three regions (Simas and Calisto, 2001).

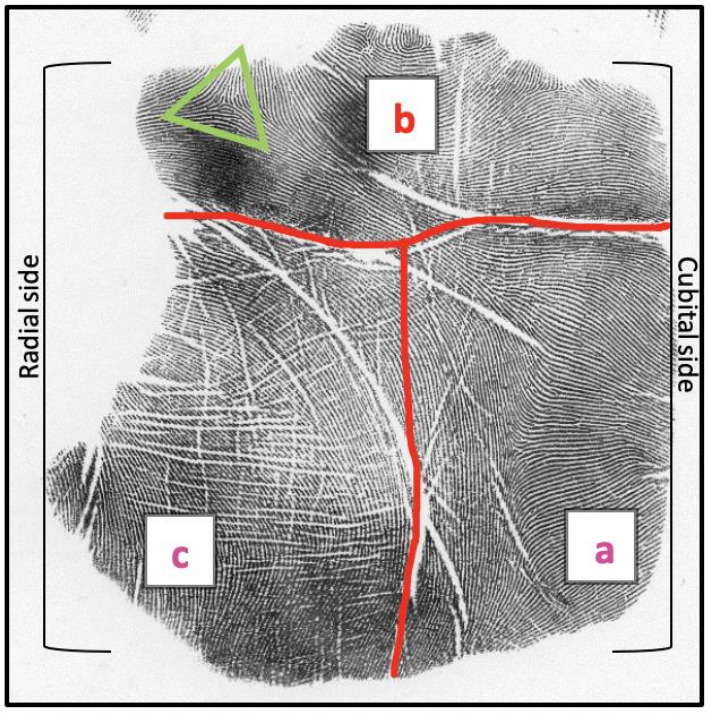


Figure 3 - Right palmprint with a tripartite division (a=hypothenar; b=superior; c=thenar)

The Olóriz System of Classification encompasses four quiroscopic types per each eminence region (Figure 4). In the hypothenar region, the type "Anucleado" means no nuclei, this is,

the non-existence of a specific figure, having ridges mostly parallel to each other (Figure 4.a). The type "Bucleado" has a "bucle" figure (loop) with its opening directed to the radial or cubital side of the palm (Figure 4.b). The type "Duplo Bucle" presents a twinned "bucle" figure (Figure 4.c) and the type "Verticilo" is characterised by a whirlwind (Figure 4.d). The superior region also has four configurations, encompassing the "Anucleado" and "Bucleado" types. Additionally, this region has the type "Piniforme" that has a "Pino" figure (arch opening to fingers; Figure 4.e) and the type "Misto" if presenting "Bucle" and "Pino" figures, simultaneously. Finally, the thenar region, that is the least frequently found as evidence, possesses the same four types of the hypothenar region.

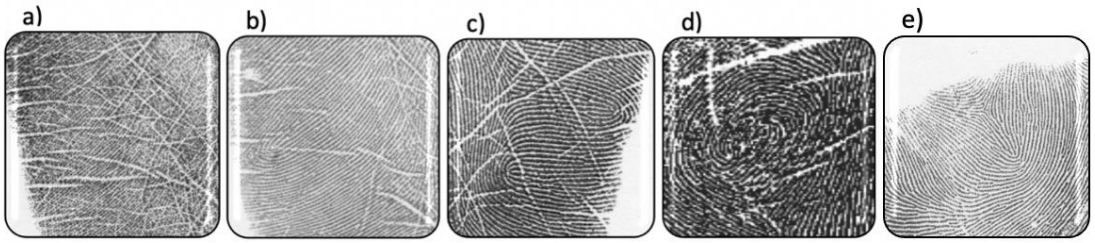


Figure 4 - Quiroscopic type classifications (a) Anucleado; b) Bucleado; c) Duplo bucle; d) Verticilo; e) Piniforme).

Quiroscopic types should also be codified, in order to construct a quiroscopic formula that easily categorize the information. The quiroscopic formula firstly presents the quiroscopic type of each region of the right hand, ending with the quiroscopic type of each region of the left hand. Hypothenar region is the first to be classified, being the thenar region the last one. Regions are codified using the first alphabetic letter of the specific quiroscopic type. Therefore, "Anucleado" type is encoded using an "A"; "Bucleado" type is encoded using a "B"; "Duplo bucle" is encoded using a "D" and "Verticilo" is encoded using a "V", "Piniforme" is encoded using a "P" and "Misto" is encoded using a "M"(Simas and Calisto, 2001).

1.2.3 MINUTIAE CLASSIFICATION

Despite the existing correspondence between the dactyloscopic type/ quiroscopic type of an evidence and the reference skin impression, in Portugal, a positive identification is only achieved when 12 equal minutiae are also found between both patterns (Table 1). In

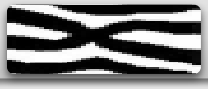
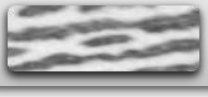
Portugal, according to the Law 67/ 2017, article 12º, "Confirmation and positive identification shall be considered to be the result of a comparison between two samples which establishes the existence of 12 common characteristic points without any divergence" (República, 2017b).

Minutiae consist in morphological peculiarities of the papillary ridges, whose disposition, form and relative position are always the same in all dactylogram or quirogram impressions from the same part of the hand. Minutiae can be very variable and may assume several forms, being read clockwise (Simas and Calisto, 2001).

Dactylograms have around one hundred minutiae and, since the palmar area is significantly higher, the quantity of minutiae that is represented is also higher, being about nine hundred (Correia, 2008). However, the number of the coincident minutiae that is needed to perform a positive identification is also twelve. Thus, the Law 67/ 2017 is applied independently of the source of the lophoscopic trace (República, 2017b).

Although there are those who argue that the influence of heredity may exist with respect to the formation of similar figures, there is no indication of this hereditary influence as regards to minutiae (Tao et al., 2012).

Table 1 - Minutiae classification (types mainly considered in Portugal).

	**Abrupta** Ridge ends abruptly, without continuity.
	**Bifurcação** One ridge divides into two.
	**Convergência** Two ridges merge in one.
	**Interrupção** Ridge ends but appears again in the same direction.
	**Pincel** One ridge branches in three (rare).
	**Olhal** Ridge forks and, without interruption, converges continuing its course.
	**Secante** Two papillary ridges converge and fork immediately, without interruption.
	**Tranversal** Ridge diverge from its path to give place to another that began, following it in parallel, assuming its path.
	**Desviante** Ridge ends, starting another slightly deviated.
	**Empalme** Small ridge joins two others that are parallel.
	**Ponto** Small isolated fragment of a ridge, having the same width and length. Usually corresponds to a pore, commonly appearing into the delta figure.
	**Fragmento** Isolated fragment of a ridge. A mini ridge, bigger than "Ponto", since it is more longer than wide.
	**Volta** Ridge reaches a certain point of its course and turns around.
	**Ramo** Small ridge that grows from another ridge, following it in a parallel way. Very similar to the "bifurcação" however the union to the 'mother' ridge is made by a small semicircle instead of an acute angle.

1.2.4 DEVELOPMENT TECHNIQUES

In several forensic investigations, latent skin ridge marks need to be developed in order to be visible, allowing the comparison between a questioned mark and a reference print. There are a huge diversity of techniques and methodologies for this purpose (Table 2), being simple (easy to use), rapid and economical. At the time of choosing the technique to be

used, the context and some features have to be taken into account. The lophoscopic expert should know when the event took place to perceive the approximate age of the possible latent marks. Additionally, the nature of the surface where evidence can be, should be evaluated (smooth or rough and/or porous, nonporous or semi-porous). The colour of the surface is also important, since there is a need to obtain a contrast between the surface and the colour of the development agent. Humidity state has also to be considered since some agents are incompatible with water (Yamashita and French, 2011).

Although skin impressions are mainly studied analysing the ridges pattern that is visible after using development agents, there are new study trends that analyse the biochemical composition of the fingerprints, not only to help in the identification of individuals, but mainly to try to ascertain, scientifically and accurately, for how long the evidence is on the surface (Girod et al., 2016; Santos, 2019; van Dam et al., 2014). At the moment, lophoscopic experts are only able to perceive and form an opinion about the dating of the latent marks by observing the "behaviour" of the used development agent. However, there is no scientific knowledge behind this type of opinion, not allowing them to register this assumption on their expert reports. Empirically, experts normally only consider that traces are "fresh" until 36 hours of existence, and "aged" with more than 36 hours (Correia, 2008).

Table 2 - Principal techniques that are used to develop latent skin ridge marks, their mechanism of action and the nature of the surface where they should be applied.

Techniques	Reaction type	Surface
Powders	Physical	Clean, smooth, nonporous surfaces ex.: glass, porcelain
Magnetic Powders	Physical	Porous and nonporous surfaces ex.: metal, glass, vinyl
Ninhydrin	Chemical	Porous surfaces ex.: paper, plaster
1,8-diazafluoren-9-one (DFO **1,1 Indanedione** **Dimethylaminocinnamaldehyde (DMAC)**	Chemical	Porous surfaces ex.: paper, thermal paper
Silver Nitrate	Chemical	Porous surfaces ex.: plastics, wood (light colour), paper
Iodine Fuming	Chemical	Porous and nonporous surfaces ex.: wood, smooth walls
Crystal Violet	Chemical	Adhesive tapes, clay, metals with little coloration
Small Particle Reagents (SPR)	Chemical	Nonporous rough surfaces ex.: keyboard, glass, plastic, dashboards
Cyanoacrylate Fuming	Chemical	Plastic, treated woods, glass, metals
Sudan Black	Chemical	Nonporous surfaces ex.: glass, metal, candles

1.3 FORENSIC GENETICS

Forensic Genetics consists in the study of nucleic acids, mainly the deoxyribonucleic acid (DNA), aiming to identify the donors of different biological traces that can be found at crime scenes or, with the aim of identifying missing persons or victims of accidents and mass disasters (Funabashi et al., 2009). In this context, DNA is commonly extracted from biological evidence such as blood, hair or semen. However, genetic expertise can also be applied when finger or palm marks do not have an identifying value, trying to extract DNA from cells that are mixed with their sebum and sweat and that can lead to the obtention of genetic profiles and the identification of individuals (Alessandrini et al., 2003; Parsons et al., 2016; van Oorschot and Jones, 1997).

DNA is a polymer formed by repetitive subunits named nucleotides, being their basic structure a nitrogenous base, a sugar (deoxyribose) and a phosphate group (Martins and Carvalho, 2008). This molecule is the biological structure that contains genetic information, being mostly located at nucleus (nuclear DNA) of eukaryotic cells, although also being

present in the cytoplasm, into the mitochondria organelles (mitochondrial DNA), the latter representing approximately only 1% of the total DNA (Pinheiro, 2008).

The human genome (complete set of genetic material) is usually divided into coding and noncoding sequences. Protein-coding sequences represent a small part of the human genome, although being the most studied component due to their importance, mainly in the clinical context. Non-coding DNA regions, the usual target regions for forensic investigations, are composed by different types of nucleotide sequences (tandem sequences, pseudogenes, introns, gene regulatory sequences and the majority of transposable elements), which represent almost 90% of the DNA of an individual (Panneerchelvam and Norazmi, 2003). About 97% of the non-coding DNA are highly polymorphic tandem repetitive sequences, being low complex multiple adjacent copies of variable lengths, that have been used for discrimination between individuals (differences in the nucleotide sequence or differences in size/ number of repetitions) (Pinheiro, 2010; Trent, 2012).

1.3.1 HUMAN IDENTIFICATION AND DNA ANALYSIS

Since Humans share most of their genome (99%) (Auton et al., 2015), in order to perform personal identification, attention should be paid to the polymorphic DNA sequences that are normally unique and different between individuals (Panneerchelvam and Norazmi, 2003). Genetic polymorphisms are highly variable regions of the genome (with a frequency higher than 1% in population) and different polymorphism types can be used for human identification (Singh, 2001). Into the genetic arena, the first technique that was discovered and used to distinguish individuals, by nuclear DNA typing, was the Restriction Fragment Length Polymorphism (RFLP). This technique involves the action of restriction enzymes which recognize specific DNA sequences, cutting the molecule in specific positions. From the action of these enzymes, recognizing or not recognizing their target regions, different length DNA fragments are produced, the result being easily observed by performing an agarose gel electrophoresis (Pinheiro, 2010). Although initially proved to be a promising technique for forensic Genetic investigations, RFLP are no longer used due to the inherent disadvantages namely, the considerable amount of needed DNA to perform the analysis

(20-30 ng) and the significant time consuming (Panneerchelvam and Norazmi, 2003). In fact, and additionally, through the application of this technique it is possible to perceive the existence of Single Nucleotide Polymorphisms (SNPs), since the failure of recognition by the restriction enzymes normally implies a variation in the DNA sequence. Thus, SNPs arise when there is a stable modification of a nucleotide, being this type of polymorphism extremely useful to distinguish between monozygotic twins and within ancestry studies and lineage identification. Nowadays, SNPs are mostly used as complementary information to distinguish and identify no related persons, being the Short Tandem Repeats (STRs) the preferred polymorphism type in forensic genetic investigations (Carvalho, 2010). STRs are a subtype of a broader group of polymorphisms, the Variable Number of Tandem Repeats (VNTRs), that are variations related with DNA length. VNTRs can be classified as minisatellites or microsatellites (STRs), the former being composed by adjacent units of 8 to 100 base pairs and the latter being composed by adjacent units of 2 to 7 base pairs (Pinheiro, 2010). The number of repetitions of these units vary among individuals and within the same individual, between the same pair of chromosomes. STRs with tetranucleotide repeats are the most commonly used in Forensic Genetics for human identification. Their alleles have very similar and small sizes (five to thirty repeats), being the small size an advantage when analysing degraded samples since even in this conditions STRs are extremely stable (Alonso et al., 2001). Normally, to assign an identification, several STRs are considered aiming to establish a genetic profile within which single and individual characteristics will be presented (except in monozygotic twins). Actually, in Portugal, the mandatory standard number of STRs markers analysed when performing forensic DNA typing is a set 8 STR loci (República, 2009).

Most of the forensic genetic analyses are executed in autosomal nuclear DNA, although DNA from mitochondria (mtDNA) and DNA from Y chromosome is also very useful to establish the individual lineage when trying to identify individuals (both apparently have only one-parent origin). When it is suspicious that the nuclear STRs approach will not be effective, for example due to high degradation and/ or low DNA amount, analysing mtDNA is an excellent alternative since it has 500 to 1000 copies per cell (Geada, 2010). These two ancestral markers are very useful in identifying persons through kinship relationships. However, despite it has always been assumed that mtDNA have only maternal origin, recent studies contradict this theory and confirm the biparental inheritance (Luo et al., 2018).

Nowadays, DNA analysis within the forensic scope, goes through a complex process that starts by the collection of the biological matrix, following by the DNA extraction from it, also isolating the nucleic acid from all other cell components. For that, an appropriate methodology of extraction should be selected (*e.g.* organic extraction, solid-phase extraction). Forensic DNA is often limited in quantity and quality thus, after extraction and purification, and in order to ensure the needed and correct amount to obtain a complete genetic profile, the existent DNA has to be amplified through a Polymerase Chain Reaction (PCR). The classic PCR is a technique that allows *in vitro* replication of small quantities of DNA, providing sufficient amount (billion copies) of one genetic marker of interest (Butler, 2012). However, when aiming to construct human genetic profiles, several targets with different sizes are amplified simultaneously in the same run, using different and specific primers, occurring a Multiplex PCR. Three major phases are involved in a PCR reaction, being the denaturation of the DNA strands, the first. Subsequently, occurs the annealing of the primers to their targets and finally the extension of the complementary DNA strand. The reaction conditions (time and temperature) and the number of cycles must be set in accordance to an optimized protocol or kit instructions. Normally, to ensure that multiplex results are suitable, an evaluation on the quantity and quality of the extracted DNA is previously performed in order to be able to set the sample to the already known ideal quantity of DNA to input. This quantification is exactly obtained by performing other type of PCR, the Real-Time PCR (qPCR). qPCR is other variation of the conventional PCR technique with the advantage of allowing to know the actual DNA quantity at the time that amplification is occurring. In this process, primers are labelled with fluorescence and results can be monitored at each cycle by measuring the fluorescence, since the signal intensity is proportional to the amount of DNA being amplified (Butler, 2010). At the end of the multiplex PCR, amplified sequences are analysed by capillary electrophoresis producing the whished genetic profile (Pinheiro, 2010). This last stage comprises DNA separation and detection. Based on the same principles of an agarose gel, DNA molecules navigate through capillarity towards the positive electrodes, being separated based on their size, the bigger DNA molecules being slower than the small ones. Fluorescence detection can also be used to improve sensitive and selectivity (Butler, 2012).

It is important to be aware that independently from the type of DNA that is used to perform human identifications, when dealing with the collection of biological samples and DNA analysis, several aspects have to be considered. Forensic samples require specific treatments, complying a set of specific rules included in the chain of custody (all requirements have to be met). The aim of the chain of custody is to preserve the integrity of samples that can possibly be used as evidence in court. The fill of all forms, during all stages, has to be ensured in order to avoid possible accidental sample contamination or loss, or even intentional exchange, or replacement. Any failure within the chain of custody may result in an inadmissible court evidence (Pinheiro, 2008).

1.3.1.1 LOW COPY NUMBER DNA - TOUCH DNA - PERSPECTIVES FOR NEW GENETIC MARKERS

Despite of all the existent knowledge, necessary for the correct establishment of genetic profiles trough STRs analysis, in some types of forensic biological samples, mainly derived from crime scenes, there is a huge difficulty of achieving complete DNA profiles, the use of short sequences not being enough to overcome the few DNA initial amount. When DNA within the biological evidence is of low quantity, usually less than 100pg, the sample is assumed as Low Copy Number (LCN) DNA. Examples of LCN samples are hair without root, bones, teeth and lophoscopic traces, where only few skin cells are present, being the DNA extracted from the latter named as touch DNA (Aditya et al., 2011).

In order to overcome this difficulty of obtaining complete genetic profiles, some approaches to LCN DNA methods have been applied, including increasing the number of PCR cycles. However, this approach also involves an increased risk of contamination and the risk of occurring some profile issues (technical artefacts) such as Allelic Dropout (ADO; one or more alleles not present), Locus Dropout (LDO; no allele displayed in a given locus) and stutter peaks (peaks that occur immediately before or after a real peak) (Kloosterman and Kersbergen, 2003). The achievement of complete genetic profiles in vestigial and/ or degraded samples is, even by using optimized methods to characterize STR markers, a difficult feat. Thus, researchers have been concerned about this problem assuming the need for new genetic markers that should be even more sensitive than the ones that are, nowadays, routinely used (STRs). Additionally, these markers should also have a remarkable

power of discrimination through small size PCR products, having a good and fast technical feasibility (Souto, 2010). Since genomic variability exists in many structural forms, attention is being paid to other DNA repeat sequences, the Transposable Elements (TE) (Figure 5).

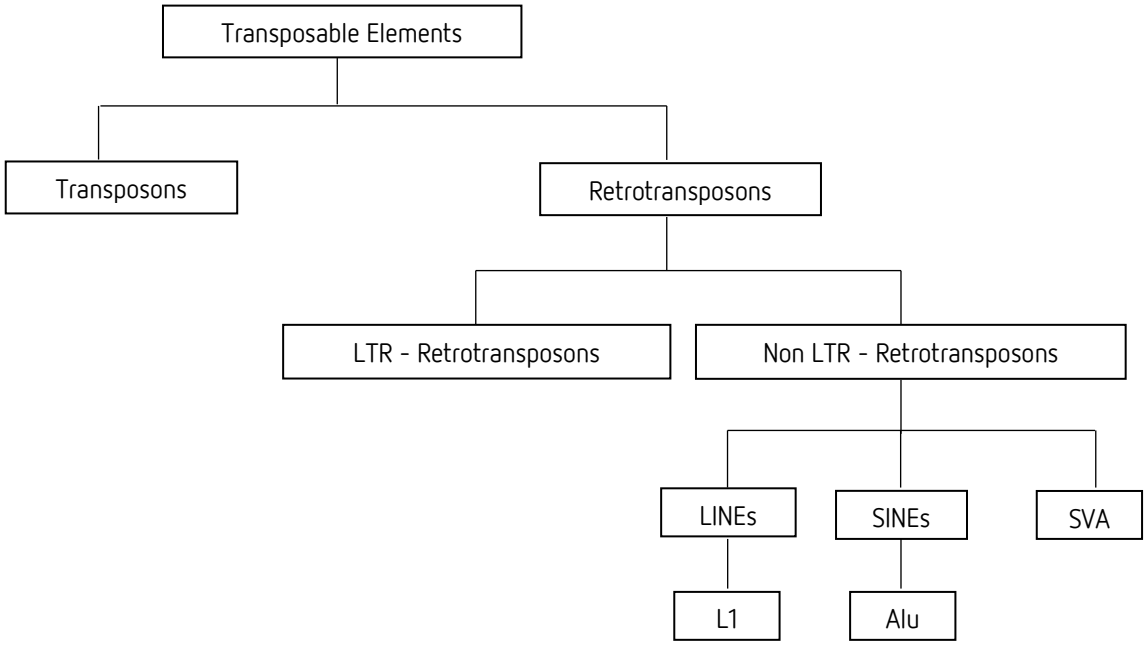


Figure 5 - Schematic representation of Transposable Elements.

Almost 45% of the human genome is composed of TE, DNA sequences that can move to new positions within the same chromosome or even to different chromosomes (Griffiths et al., 2015) . TE mechanistically produce, in the local of integration, genetic alterations that according to the transposition process can result in insertions, excisions, duplications or translocations. According to the nature of their mobilization intermediary, TE are subclassified as transposons or retrotransposons (Muñoz-López and García-Pérez, 2010). Transposons, through enzymes action, are cut from the main DNA molecule (forming an insertion site), being reintegrated into new genomic sites (cut and paste mechanism). They are mainly present in simple organisms, not being currently active in the human genome, representing around 3% of it. The vast majority of human TE are retrotransposons, that also rely on a cut and paste mechanism, although amplifying themselves through RNA intermediates that are reversed transcribed into complementary DNA (cDNA) before being again inserted into the main DNA molecule. Retrotransposons move similarly to the mechanism of a retrovirus life cycle and are subclassified into two groups, the Long

Terminal Repeats (LTRs), with an action limited in humans, and Non-LTR retrotransposons being mostly active in the human genome (Ayarpadikannan and Kim, 2014; Cordaux and Batzer, 2009; Finnegan, 2012). Non-LTR retrotransposons instead of having a structure named long terminal Repeat, possess a terminal poly-A tail, being again subdivided in three subgroups: the Long Interspersed Nuclear Elements (LINEs, *e.g. L1*), the Short Interspersed Nuclear Elements (SINEs, *e.g. Alu*) and the SINE-R/VNTR/Alu (*SVA*) elements (LaRue et al., 2012). *L1*, Line type retrotransposons, are the dominant retrotransposons in the human genome, being autonomous and encoding the proteins necessary for transposition, particularly the ORF2p, a reverse transcriptase responsible for the reverse transcription of the RNA retrotransposon form to cDNA (Hancks and Kazazian, 2010). Despite their abundance most of them do not remain intact (transpositional inactive *L1*), the full-length *L1* elements having very long sizes (6 Kb). SINEs are the second most abundant TE class in the human genome, having less than 400 bp and being non-coding DNA regions (Snustad and Simmons, 2008) . *Alu* elements are primate specific SINEs (approximately 300 bp), representing 10% of the human genome and being non-autonomous, depending on the enzymatic machinery of the complete *L1* for transposition (Cordaux and Batzer, 2009; Hormozdiari et al., 2011). Some *Alu* subfamilies only became active when human lineage diverged from its common ancestor, the nonhuman apes, being responsible for the production of new *Alu* insertions (exclusive of humans) that are stable and polymorphic (*i.e.* reaching 50% heterozygosity in all major populations), being the absence or presence of the new *Alu* insertions, the *Alu* alleles. The absence of the allele is considered the ancestral allele and, once new insertions are present, they are stable not being removed. Due to short length and polymorphic forms, *Alu* elements can be potentially used as DNA markers in recent forensic genetic analysis (Ayarpadikannan and Kim, 2014; LaRue et al., 2012). Like *Alu* retrotransposons, SVA insertions are about 2700 copies in the human genome reference sequence. SVA are composite retrotransposons being also polymorphic and non-coding/ non-autonomous, although having a considerable full-length (until 4000 pb) (Hancks and Kazazian, 2010).

Recent approaches have been testing the possibility of performing genetic identification through retrotransposons, mainly *Alu* elements, using these Insertion-Null markers (INNULs) (Martins and Carvalho, 2008). The use of this type of markers can raise ethical concerns since despite being non-coding regions, their presence or absence can have

impact in gene expression, supplying the persons that have access to genetic profiles with data considered sensitive. Nevertheless, they have several advantages when compared with the commonly used STR markers: the bi-allelic retrotransposons nature, per marker, allows a simpler analysis and a simpler interpretation of results; genetic profile interpretation is also easier since it is not possible to observe stutters related to polymerase slippage; as SNP polymorphisms, retrotransposons have low mutation rates being quite useful for establishing lineage in cases of missing persons or victims of mass disasters; once retrotransposons do not present homoplasy nature they can also be used to identify individuals of specific population groups and they are also useful to identify genetic ancestry (LaRue et al., 2012; Sinha et al., 2015; Snustad and Simmons, 2008). Finally, it is also important to mention that when typing INNULLs in samples with DNA mixtures performing conventional capillary electrophoresis, limitations are assumed. However, these limitations can be overcome when having reference samples or with the analysis of samples in Next Generation Sequencing platforms (Sinha et al., 2015).

II. AIMS

Crimes related with car assaults and carjacking have been considerably increasing around the world (StolenCars24, 2017), therefore there is an urgent need to correctly pinpoint individuals involved in such events. Some type of inner car surfaces, such as synthetic skin coating car steering wheels, usually impose additional difficulties to perform fingerprint analysis. Hence, and since fingerprints can be also a source of DNA, the current work primarily aims to assess those difficulties, also testing the compatibility and complementarity of fingerprint and DNA analysis, in order to successfully achieve the unequivocal identification of individuals. Consequently, the current work will allow to test and deepen knowledge on the practical use of recent genetic retrotransposon-based kits (InnoQuant® HY-R; InnoTyper® 21) that were specially developed to analyse biological forensic traces with few and degraded DNA. These genetic kits, despite their developmental validation (Brown et al., 2017; Loftus et al., 2017), they were already internal validated to be used in real cases in the Laboratório de Polícia Científica da Polícia Judiciária (LPC-PJ) (Martins et al., 2018). Acting in a mock scene, it is the final aim of this work to scientifically contribute to the settlement of more suitable protocols of action to be applied when dealing with real crime scenarios, even if possibly implying the need for future Portuguese legislative modifications.

3.1. VOLUNTEERS RECRUITMENT AND ETHICAL ISSUES

The volunteer's group consisted in twelve Caucasian donors, that were selected mainly accordingly to an age criterion, having more than 18 years old since, in Portugal, it is the minimal age to legally have a driving licence. The criterion of sex was also applied, being 50% females and 50% males. No more intentional variables were added.

3.2. ETHICAL ISSUES: VOLUNTEER´S PRIVACY AND CODIFICATION

The privacy of volunteer's data was assured, according to the European Union General Data Protection Regulation (Parliament, 2016). Volunteers were asked to fill out an identification sheet (Attachment 1), including information on their age, sex, profession, personal contacts and hand hygiene habits. Since some personal information of the volunteers can influence their sebum, sweat and cells release, some issues are important to be taken into account in order to better understand possible results. Each volunteer´s identification sheet was codified through a number, assuring that solely the Principal Investigator (PI) had access to the identity data of each sample donor and only during the execution of the project. Additionally, an informed consent (Attachment 2) was signed out by the donors, allowing the use and processing of the collected data. A confidentiality agreement was provided in the same document, specifying and assuring that their biological data were only to be used in the current project. All documents, previously to be filled in, were approved by the University Institute of Health Sciences (IUCS) Ethics Committee (Attachment 3).

It is important to highlight, previously to the description of any practical work, that only the PI had access to the identity data and numerical identification of the volunteers, until the end of the experiment. The numerical data were only disclosed after the interpretation of the results, in order to avoid bias. Additionally, at each moment, volunteers were sampled according to their temporal availability, being the samples sequentially codified through the use of letters, from A to L. Thus, the use of the same capital letter to codify samples at different moments, did not mean the same volunteer. However, the PI was always present to perform the correspondence between letters and number identification, saving the

information (Attachment 4). Additionally, two different researchers assisted different moments within the collection of lophoscopic and genetic samples, to also ensure the elimination of bias, being the remaining laboratory work only executed by the same researcher to avoid differences in results due to operator variable. Enlightening, the volunteer's codification with numbers assured the privacy of the identity dada and the codification with letters assured the inexistence of bias in the interpretation of the results.

3.3. CREATION OF VOLUNTEER´S DATABASES

3.3.1. LOPHOSCOPIC DATABASES

3.3.1.1. FINGERPRINT DATABASE

The collection of fingerprints was performed according to the Portuguese Law nº 67/2017 of August 9, article 4 (República, 2017b). Thus, all fingers of each volunteer were printed using a proper ink pad (Figure 6), being posteriorly pressed on a dactyloscopic bulletin similar to the model that is used by the Polícia Judiciária (PJ) (Attachment 5). Two different sets of fingerprints were collected on the same document, rolled and plain, being the rolled prints collected at the front of the bulletin and the plain prints collected in the reverse side. The collection of rolled fingerprints started by the right thumb (first finger) and ended in the left little finger (tenth finger). When collecting fingerprints, the wrist anatomy was respected rolling fingers from the centre towards the outer part of the body. In each finger, complete rolled prints represent the skin pattern from nail to nail and from the top of the distal phalange until the flexion fold.

Figure 6 – Ink pad for fingerprint collection (Sirchie).

The collection of plain fingerprints was performed just pressing the fingers against the bulletin, starting by the last four right fingers at the same time, and then the right thumb, posteriorly repeating the same procedure with the left hand. In each finger, complete plain prints represent the skin pattern from the top of the distal phalange until the flexion fold. Between the collection of rolled and plain fingerprints, volunteers washed their hands to remove the excess of ink in order to do not damage the consecutively collections, since the quality of the pattern obtained is mainly influenced by the used ink (quality and quantity) and the executed pressure. The presence of water can also influence the pattern quality thus, hands were completely dry before performing new collections. It was also verified that no type of contamination was on the ink pad, in order to avoid the impression of fake patterns. It is important to notice that, due to the lack of experience on collecting fingerprints, four bulletins of each volunteer were filled in for further selection, based on the printed patterns quality. Hands were also washed between different bulletin collections. Consecutively, all bulletins were identify using a capital letter from A to L, and after having selected the best dactyloscopic bulletin per each volunteer, all fingerprints were classified and codified according to their dactyloscopic type, using the Olóriz System of Classification. After double confirmation (second evaluation executed by a Senior Forensic Expert), the dactyloscopic formulas were inserted in the respective bulletins.

The predominance of the dactyloscopic types of the population of the current work was evaluated, and the best dactyloscopic bulletin of each volunteer was scanned and inserted

into Automated Fingerprint Identification System (AFIS), in a temporary file, to act hereafter as a fingerprint database/ reference samples. Normally, the dactyloscopic type is the first filtering when using AFIS to compare fingerprints.

Additionally, in order to evaluate the minutiae predominance of the population of the current work, dactyloscopic bulletins were copied and a rectangular sampling area was applied on each fingerprint. The size of the sampling area (1.6 cm wide; 1.9 cm length) was defined according to the smallest fingerprint of the twelve volunteers (a female, and the position where it was set depended on the dactyloscopic type of each fingerprint, having the care to select the areas that typically have more minutiae. Thus, in the "Adéltico" type, the rectangle included the centre of the fingerprint; in the "Monodéltico" type, the rectangle included the delta figure and the nuclear system and in the Bidéltico"/ "Polidéltico" type, the two deltas or, at least, the external delta and the nuclear system, where included in the rectangle. Relevant is to add the information that, when trying to encompass the desirable targets, if empty areas were also included, an adjustment of the area selection was always carried out to be able to correctly compare results. The selection of the fingerprint area that should be encompassed into the rectangle and the avoiding of empty spaces adjusting the sampling location, is similar to what AFIS does, when comparing fingerprints.

Figure 7 - Magnifying glass to analyse fingerprints, palmprints and lophoscopic evidence.

3.3.1.2. PALMPRINT DATABASE

The collection of palmprints was performed according to the Portuguese Law nº 67/2017 of August 9, article 4 (República, 2017b). The law states that plain palmprints should be collected, as well as palmprints in the hand position assumed while writing. However, due to the context of the present work, the latter were not collected. Thus, two plain palmprints were impressed with the help of a proper set of materials (Figure 8), consisting in a metal support were some tube ink was spread, using a roll. Posteriorly, the palm hands were inked with the roll and pressed on a quiroscopic bulletin, similar to the model that is used by the PJ (Attachment 6). The collection started with the right palm (front of the bulletin), placing the roll at the basis of the palm (close to the wrist) and rolling up in direction to fingers, including the flexion folds. Same procedure was repeated with the left hand (reverse side of bulletin). To the image of what was executed when constructing the fingerprint database, four bulletins of each volunteer were filled in for further selection, and same cares were taken in order to obtain the best results.

Consecutively, all bulletins were also identified using a capital letter from A to L, and after having selected the best quiroscopic bulletin per each volunteer, all palmprints were classified and codified according to their quiroscopic type, using the Olóriz System of classification. Again, after double confirmation (second evaluation executed by a Senior Forensic Expert), the predominance of the quiroscopic types of the population of the current work was evaluated and the best quiroscopic bulletin of each volunteer was scanned and inserted into AFIS, in a temporary file, to act hereafter as palmprint database/ reference samples.

Figure 8 – Set of materials for palmprint collection.

3.3.2. GENETIC DATABASE

The analysis of forensic biological samples requires laboratory good practices to prevent and avoid any undesirable sample contamination that can hamper the interpretation of results or nullify the value of evidence in court. Therefore, most of the laboratory work dealing with deoxyribonucleic acid (DNA) was performed into a laminar flow chamber (Figure 9), previously and consecutively disinfected and sterilized with sodium hypochlorite 3%, ultrapure water, ethanol 70% and 30 minutes of ultraviolet lights, sequentially. Most of the used materials were firstly autoclaved for sterilization and all the materials were previously disinfected with ethanol 70% before being introduced into the chamber. Clean lab coats and gloves were used along the whole process, and the latter were also sprayed with ethanol 70% before arms introduction into the flow cabinet. Aliquots of all reagents required for DNA extraction were performed.

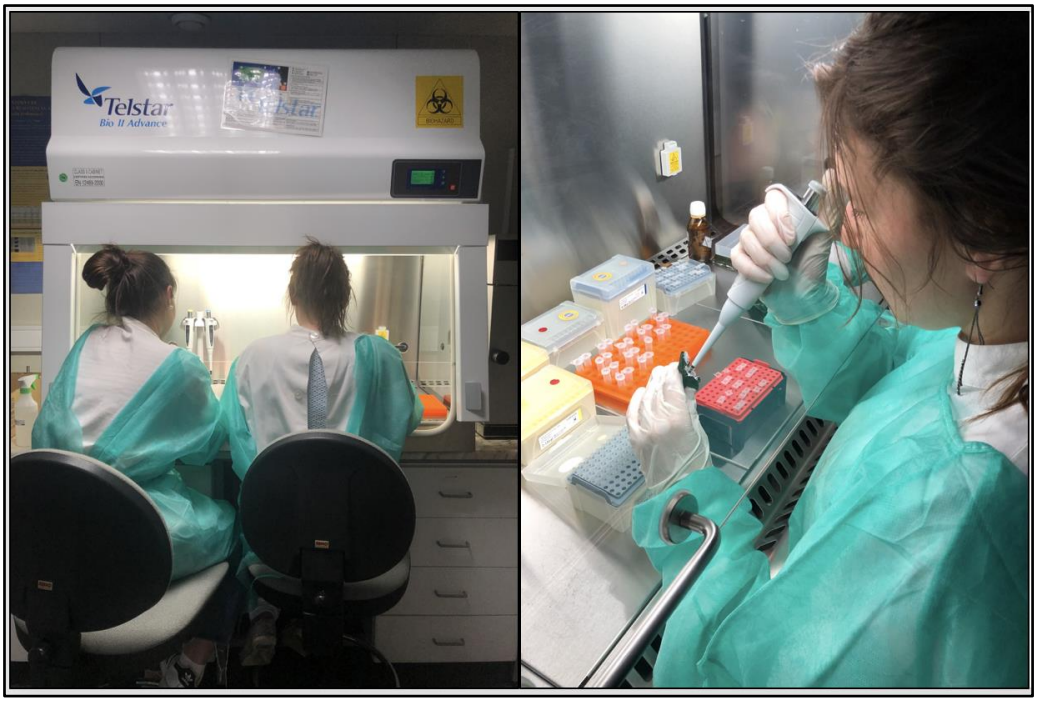


Figure 9 - Laboratory work under laminar flow chamber conditions.

3.3.2.1 COLLECTION OF BIOLOGICAL SAMPLES

The collection of biological samples was performed according to the Portuguese Law nº 90/2017 of August 22, article 10 (República, 2017a). Thus, it was executed using a non-invasive method, by scraping the buccal mucosa with a sterile and dry cotton swab (Figure 10).

Figure 10 - Collection of biological samples for constructing DNA database: a) harvesting cells from the buccal mucosa; b) sterile cotton swab.

Two buccal swabs were collected on each volunteer, firstly on the inner surface of the right cheek and then on the inner surface of the left cheek. In order to standardize the collection methodology, scraping was carried out rolling the swab for sixty seconds, on each cheek. Each swab was put into an *Eppendorf* previously identified using the respective capital letter from A to L, adding also the small letter "a" to the swab of the right check and the small letter "b" to the swab of the left check.

3.3.2.2 DNA EXTRACTION

Despite of the toxicity and the significant time consuming, due to the context of the current project and based on the need of having a high DNA profitability, the phenol-chloroform methodology (a liquid-liquid extraction) was selected. The used protocol was optimized from the protocol that was adopted by Cainé (Cainé, 2010) to extract DNA from the gastric juice of fly larvae. The main modifications to this protocol were the adjustment in the lysis buffer volume (400 µL); the addition of DTT and Proteinase K apart from the lysis buffer

and only at the moment of extraction; the addition of 400 µL of water saturated *n*-butanol to the aqueous phase, and the replacement of vortex by clinical rotator after the lysis buffer action.

In this way, after swabbing for one minute, on each cheek, each swab was placed into a previously identified and autoclaved *Eppendorf* having inside 360 µL of lysis buffer (stock solution: 10 mM Tris-HCl at pH 8.0; 2 mM EDTA; 10 mM NaCl; 1% SDS) plus 20 µL of DTT and 20 µL of proteinase K that were added just before starting the protocol execution. The plastic handle of each swab was cut, and each *Eppendorf* was closed. Posteriorly, each sample was vortexed for one minute to release its cells from the cotton swab fibbers and placed into a water bath at 56 °C (ideal temperature for proteinase K performance) about three hours, being posteriorly vortexed again for five seconds, discarding the swab. Then, each sample was centrifuged (Spectrafuge™ 24D Digital Microcentrifuge) at 14000 rpm, for three minutes, and the upper phase was transferred to a new *Eppendorf*, adding 400 µL of phenol: chloroform: isoamyl alcohol (25:24:1). Each sample was then placed on a clinical rotator (Fisher scientific 64724) for ten minutes and centrifuged at 14000 rpm for five minutes. The phenol: chloroform: isoamyl alcohol mixture leads to the cellular components isolation by obtaining a separation of phases. In the lower organic phase, proteins are dissolved in phenol and lipids are dissolved in chloroform. The upper aqueous phase presents the DNA (McKiernan and Danielson, 2017).

Subsequently, each aqueous phase was transferred to a new *Eppendorf*, being added 400 µL of water saturated n-butanol to remove residual organic solvents. Each sample was then mixed in a clinical rotator for five minutes and centrifuged one minute at 13000 rpm for separation of phases. At that point, each upper phase was discarded, adding 600 µL of cold absolute ethanol to the lower phase to precipitate DNA. Each sample was again mixed in a clinical rotator for fifteen minutes and then placed at -20°C for thirty minutes, to complete DNA precipitation. Time elapsed, each sample was centrifuged at 13000 rpm for five minutes to concentrate DNA at the bottom of each *Eppendorf*. Ethanol was then removed carefully with a micropipette and each sample was allowed to dry for thirty minutes / until complete evaporation. The complete ethanol evaporation is very important since it may cause inhibition of the Polymerase Chain Reaction (PCR). At the end, 25 µL of autoclaved deionized water (dH2O) were added and each reference sample was stored at -20°C, until being quantified by Real-Time PCR (qPCR).

3.4. MIMETIZATION OF A REAL-LIFE SCENARIO DRIVING A CAR

3.4.1. CAR INNER SURFACE AND MAKE SELECTION

According to the needs of a person when driving a car, the inner surfaces that are frequently touched are the steering wheel, the speed lever, the radio and the rear-view mirror. Among those surfaces, practical experience of the lophoscopic experts of the PJ, demonstrated the need of improving the possibilities of identifying car drivers, based on the analysis of the steering wheels, since it is commonly difficult to achieve an identification by the development of finger or palm marks on it. Moreover, according to statistical analysis in 2017 of the European most stolen cars, Volkswagen and Audi vehicles emerged occupying the first and second position, respectively (Figure 11) (StolenCars24, 2017).

Combining the presented information and based on the availability of buying two equal second hand steering wheels of the two makes, Audi was selected.

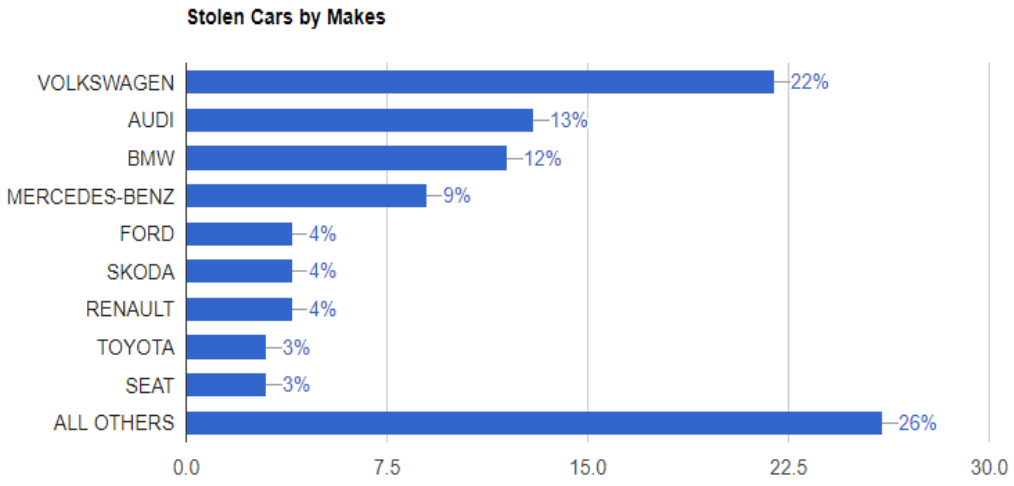


Figure 11 – Statistical analysis of the European most stolen cars, in 2017 (adapted from (StolenCars24, 2017)).

After the steering wheels purchase, they were lined using new skin, the same skin type that is also used to cover the first hand specimens, and applying a similar coating methodology.

3.4.2. DRIVING SIMULATION AND SAMPLING DESTINATION

Each volunteer went twice to IUCS facilities to simulate a real event driving a car. In those two times, volunteers were asked to sit and drive a steering wheel that was fixed on a metal structure designed and constructed proposedly for the current work (Figure 12), allowing the steering wheel to spin as it normally spins during the driving of a real car.

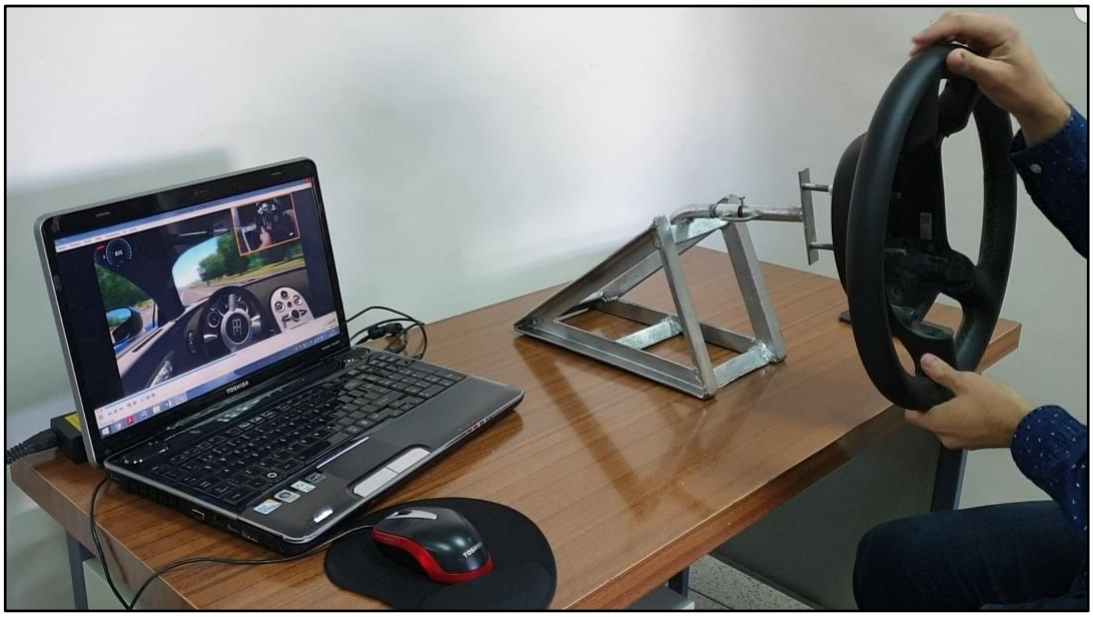


Figure 12 – Driving simulation.

To mimic a real-life scenario and to standardize the driving conditions between volunteers, they were asked to look to a driving video for fifteen minutes, executing the same hand movements of the video driver (*e.g.* removal of the volunteer´s hand from the wheel at the same time that the video driver does it).

Since the steering wheels available were only two, having only one metal support, also only 2 driving simulations were allowed to be performed per day, in both simulation times. Biological traces from the first simulation were directly used for genetic analysis, without any interference of any development agent for visualizing finger and palm marks. Biological traces from the second simulation were directly used for lophoscopic analysis, being also consecutively used for genetic analysis, in order to evaluate possible interferences of the development agent with the genetic analysis process.

3.4.3. LABORATORY PRACTICE AVOIDING STEERING WHEELS CONTAMINATION

During both simulations, between each use of the steering wheels, they were cleaned with ethanol 70% followed by ultrapure water (two repetitions). Steering wheels were then submitted to UV radiation inside a laminar flow chamber for 30 minutes in total, being the back side directly exposed for the first 15 minutes and then the front side for the last 15 minutes. Additionally, in the second simulation, and since a chemical reagent was applied on the steering wheels each time they were used, previously to the clean process already described, steering wheels were also cleaned with pure acetone followed by ultrapure water (two or more repetitions, until the chemical agent was no longer seen (Figure 13) (Risoluti et al., 2019; Turss et al., 1970).

Figure 13 - Cleaning of a steering wheel after the application of the lophoscopic development reagent.

3.5. FORENSIC EXPERTISE

3.5.1 FINGERMARK AND PALM MARK ANALYSIS

After each driving simulation, steering wheels were transported, into different police evidence paper bags (Figure 14), to the criminalistic laboratory of the PJ, in order to perform the cyanoacrylate (super glue) fuming. Among all the development techniques, cyanoacrylate fuming was selected since although being mostly used in nonporous surfaces

(Ramotowski, 2012), it is also the technique that is normally used in the PJ facilities when challenge surfaces, as synthetic fabrics, need to be analysed. Additionally, in real cases, cyanoacrylate is also applied to develop latent finger and palm marks inside cars, through the use of cyanoshots.

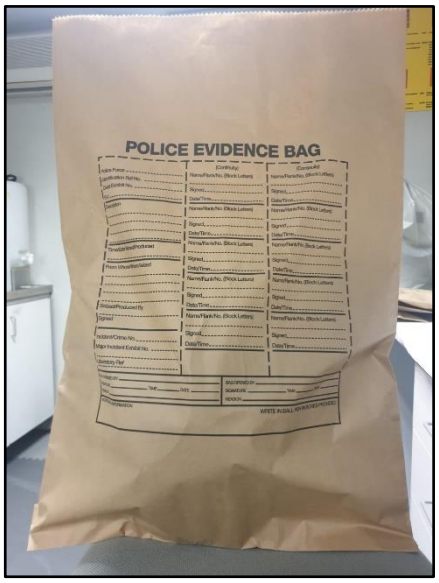


Figure 14 - Police evidence paper bag.

When developing latent finger and palm marks with this technique, inside a laboratory, a fumigation chamber is needed (Figure 15). So that, the two steering wheels were placed, in parallel, into the Projetina® fuming chamber at each time it was used. Before starting with each fuming process, three grams of cyanoacrylate were put on an aluminium container, located above the heating plate (Figure 15, blue arrow), being the chamber door closed. Humidity was set to 80% for 25 minutes. Dehydrated latent marks lead to less effective development thus, fuming under high humidity conditions can improve results (Ramotowski, 2012).

Figure 15 - Cyanoacrylate Projectina® fuming chamber: a) external view and b) internal view (blue arrow=aluminium container).

Elapsed the humidity time, the temperature was set on to 140 °C for 30 minutes. Cyanoacrylate vaporizes with the increase of temperature, subsequently polymerizing on the evidence epidermal ridges. Lastly, before removing the steering wheels, cyanoacrylate vapor was extracted for ten minutes.

Steering wheels were then visually analysed in order to observe the existence of developed traces with possible identification value. Developed traces in those conditions were then photographed for posterior lophoscopic analysis. Due to the nature of the surface where traces were developed (dark, rough and possibly semi-porous), the photographs were improved relatively to brightness and contrast, being also cut to focus attention on the specific evidence. All the evidence photographs, with possible identification value, were introduced into AFIS, to act hereafter as lophoscopic questioned samples (samples with unknown provenance). The automated system was asked to find "Hits" between these questioned samples and the previously stored databases. Due to the lack of a proper scale

(needed to use AFIS) in some good evidence photographs, some questioned samples were informatically evaluated and compared although only through the knowledge of a Senior Forensic Expert.

3.5.2 GENETIC ANALYSIS

All steps of the genetic expertise were executed after the 2 driving simulations and, excluding the DNA extraction, all of the other laboratory treatments were executed at the same time, dealing with reference and questioned samples.

3.5.2.1 COLLECTION OF EVIDENCE

Having each steering wheel placed in the metal structure, the collection of evidence was performed using two swabs. The fist swab was submerged in ultrapure water and then rolled clockwise on the right half of the steering wheel (Figure 16). The second swab was also submerged in ultrapure water and then rolled counter clockwise on the left half of the steering wheel.

Figure 16 - Steering wheel being swabbed for collection of evidence after cyanoacrylate fuming.

Each swab was put into an *Eppendorf* previously identified with the respective capital letter from A to L, plus the small letter "v" for vestigial samples without cyanoacrylate or "c" for vestigial samples developed with cyanoacrylate. Additionally, was also added the small letter "a" to the swab of the right part of the steering wheel and the small letter "b" to the

swab of the left part (*e.g.* "*Dca*": "D" meaning the fourth volunteer + "c" meaning cyanoacrylate + "a" meaning the right side of the steering wheel).
All the vestigial evidence collected from both driving simulations were assumed as questioned samples.

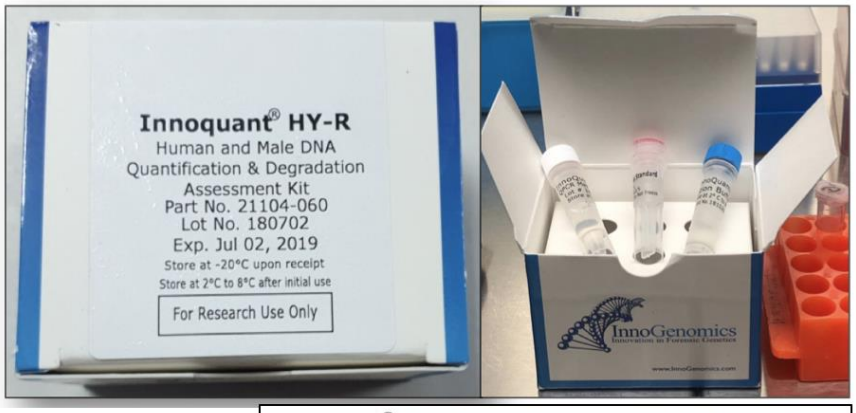
3.5.2.2 DNA EXTRACTION

Immediately after swabbing the steering wheels, each swab was placed into a previously identified and autoclaved *Eppendorf* having inside 360 µL of lysis buffer (stock solution: 10 mM Tris-HCl at pH 8.0; 2 mM EDTA; 10 mM NaCl; 1% SDS) plus 20 µL of DTT and 20 µL of proteinase K that were added just before starting the protocol execution. Posteriorly, the protocol applied for DNA extraction was the same already described for extracting DNA from buccal swabs.

3.5.2.3 ASSESSMENT OF DNA QUANTIFICATION AND DEGRADATION

Assessment of DNA quantification and degradation of the reference and questioned samples was performed through qPCR (Bio-Rad CFX96 - C1000™ Thermal Cycler; Figure 17) using the InnoQuant® HY-R Human and Male DNA Quantification & Degradation Assessment Kit (Figure 18). All samples were analysed on a single run in order to minimize variables that can hamper the interpretation of results (*e.g.* different running conditions).
When working, all the manufacturer's instructions present on the user guide of the kit were strictly followed (InnoGenomics Technologies, 2018) and, previously to the quantification of the current work samples, two experiments were performed to ensure a correct near future performance.
The assessment of DNA quantification and degradation through the InnoQuant® HY-R kit encompassed three main steps: a) Reaction and sample setup, b) Preparation of DNA standards (Stds.) and c) Cycling parameters setup and qPCR run, and started by thawing at room temperature, for at least 15 minutes, all the components.

Figure 17 - Real-Time Polymerase Chain Reaction (qPCR) Detection System (Bio-Rad CFX96 - C1000™ Thermal Cycler).



InnoQuant® HY-R Primer Mix	1 red top tube
DNA Standard, 100 ng/µl	1 clear top tube
InnoQuant® Dilution Buffer A	1 blue top tube
InnoQuant® HY QPCR Master Mix	1 white top tubes

Figure 18 - InnoQuant® HY-R Human and Male DNA Quantification & Degradation Assessment Kit (InnoGenomics Technologies; right image missing the primer mix tube).

During the process, some cares were taken: low adhesion filter micropipettes tips were used; primer mix tube, and all tubes containing it, were always protected from light due to the light-sensitive probes; primer mix tube was never centrifuged, avoiding concentration of the primers at the bottom; contents were placed at the bottom of the tubes; no bubbles were allowed to be into the tubes previously to the qPCR run.

In an effort to avoid as much contamination as possible, since the work plan included amplifying reference samples (that would have a considerable amount of DNA), and also questioned samples (that would have few DNA), the setup of the experiment started by the

latter and then the former. It was also an option to do the setup of the standards at the last moment.

a) Reaction and sample setup

The Reaction Mix (RM) was prepared considering the recommended quantities of each component (Table 3), taking into account the 72 samples, plus the 5 DNA Stds. in duplicate, 1 Non-Template Control (NTC) and some additional reactions to assure enough volume due to the loss that normally exists during the reagent transfers. Besides the negative control NTC, two other negative controls were added to the experience (not required by the kit´s manufacturer).

Table 3 - Reaction Mix components and respective quantities per sample (InnoQuant® HY-R Kit).

PCR components	Volume per Reaction
InnoQuant® QPCR Master Mix	10 µL
Nuclease-Free Sterile Water	0.3 µL
InnoQuant® HY Primer Mix	7.7 uL
Total volume	18.0 µL

Obeying to a layout previously outlined, and using 8-tube PCR strips, 20 µL of ultrapure water were added to the 2 extra negative control tubes. Consecutively, 2 µL of InnoQuant® HY Dilution Buffer A were added to the NTC tube and then, 2 µL of questioned samples developed with cyanoacrylate were also added to their tubes. Sequentially, 2 µL of questioned samples without cyanoacrylate and 2 µL of reference samples were also added to the respective tubes. Finally, the RM was vortexed and 18 µL were added to all tubes (exception of the two extra negative controls), pipetting up and down. All strips were capped and centrifuged at 1500 rpm for 1 minute.

b) Preparation of DNA standards

Firstly, the InnoQuant® DNA HY-R DN Std. stock (100 ng/ µL) was vortexed for 5 seconds and centrifuged at 3000 rpm for 15 seconds. Then, gloves were changed, and 5 serial dilutions of the DNA Std. were prepared using the InnoQuant® Dilution Buffer (Table 4). All

standard dilutions were prepared in duplicate to increase the precision of the calibration curve, encompassing Std. concentrations from 20 ng/ µL (Std.1) to 0.005 ng/ µL (Std.5).

Table 4 - Settings for preparing serial dilutions of the InnoQuant® DNA HY-R DNA Standard (InnoQuant® HY-R Kit).

Standard	Concentration (ng/µL)	Recommended Dilution Amounts	Dilution Factor
Std.1	20	10 µL [stock 100 ng/µL] + 40 µL Dilution Buffer A	5x
Std.2	2.5	10 µL [Std. 1] +70 µL Dilution Buffer A	8x
Std.3	0.3125	10 µL [Std. 2] + 70 µL Dilution Buffer A	8x
Std.4	0.0391	10 µL [Std. 3] + 70 µL Dilution Buffer A	8x
Std.5	0.005	10 µL [Std. 4] + 70 µL Dilution Buffer A	8x

Finally, 2 µL of each DNA standard dilution were added to their respective tubes, being posteriorly filled with 18 µL of the RM, pipetting up and down. All strips were capped and centrifuged at 1500 rpm for 1 minute.

c) Cycling parameters setup and qPCR run

Obeying to a layout previously outlined, all 8-qPCR tube strips were introduced on the thermal cycler of the Bio-Rad CFX96 (Figure 17). A new protocol was created into the system with the specific cycling conditions for the specific run, that started immediately (Figure 19).

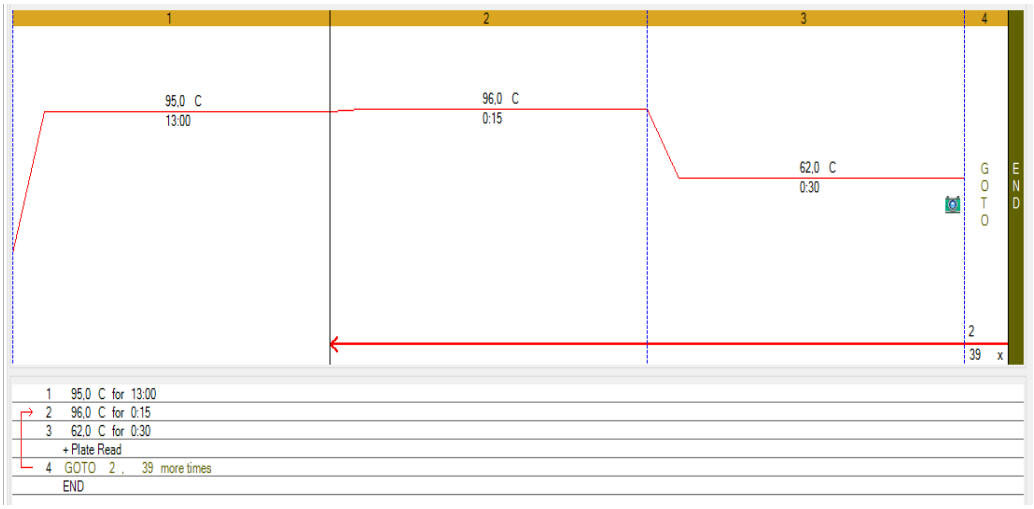


Figure 19 - Cycling Parameters for the qPCR of the InnoQuant® HY-R Kit.

3.5.2.4 DNA TYPING

DNA typing of the reference and questioned samples was performed through a multiplex PCR (Bio-Rad CFX96 - C1000™ Thermal Cycler; Figure 17) followed by capillary electrophoresis (Applied Biosystems 3500 Genetic Analyser; Figure 20) using the InnoTyper® 21 Human DNA Analysis Kit (Figure 21). Previously to any analysis, the sequencer was calibrated using a Multi-Capillary IGT 5 Dye Matrix Std. (Figure 22).

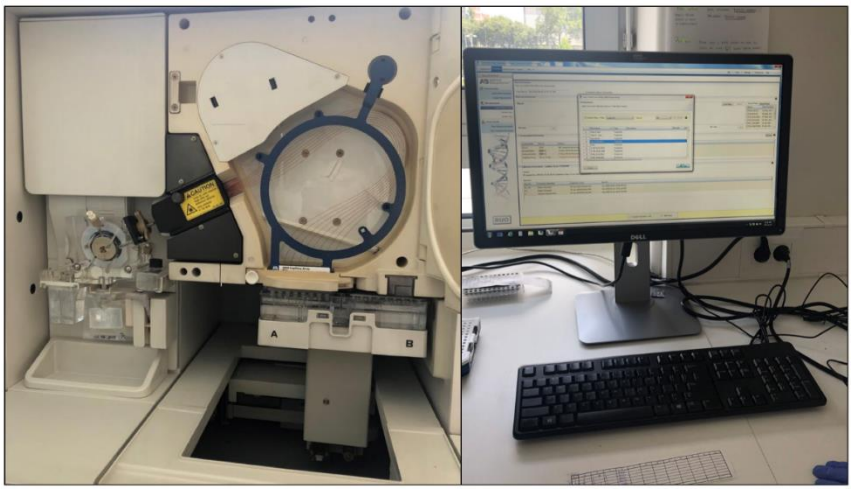


Figure 20 - Applied Biosystems 3500 Genetic Analyser.

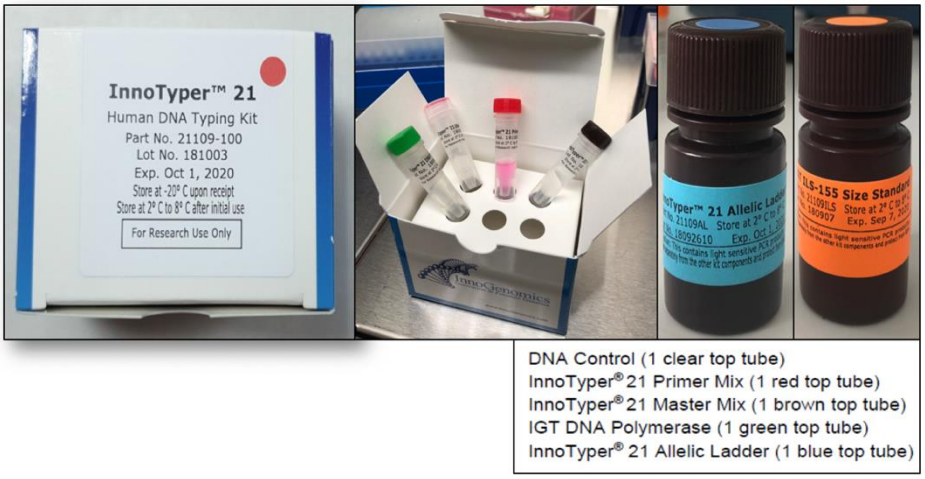


Figure 21 - InnoTyper® 21 Human DNA Analysis Kit (InnoGenomics Technologies).

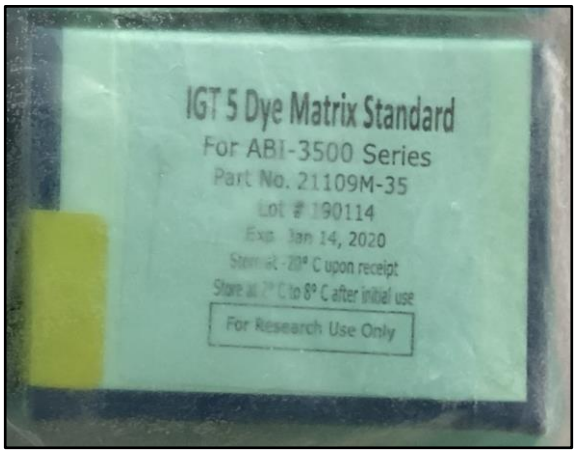


Figure 22 - Multi-capillary IGT 5 Dye Matrix Standard (InnoGenomics Technologies).

When working, all the manufacturer's instructions present on the calibration manual (InnoGenomics Technologies, 2015) and on the user guide of the kit (InnoGenomics Technologies, 2017) were strictly followed and, previously to the amplification and fragment analysis of the current work samples, one experiment was performed to ensure a correct near future performance. This experiment was also crucial to decide the best DNA quantity to input for multiplex PCR (when possible). However, it is quite important to highlight that, differently from the kit instructions, POP-7™ Polymer and 50 cm capillary arrays were used.

The DNA typing through the InnoTyper® 21 Human DNA Analysis Kit encompassed three main steps: a) Calibration setup b) Multiplex PCR and c) Capillary electrophoresis, and started by thawing at room temperature, for at least 15 minutes, all the components. During the process some cares were taken: filter micropipettes tips were used; IGT 5, primer mix tube and all tubes containing it, allelic ladder and size std. were always protected from light due to the light-sensitive fluorescent probes and dyes; matrix Stds. and primer mix tube were never centrifuged; contents were placed at the bottom of the tubes; no bubbles were allowed to be into the tubes previously to the multiplex PCR run.

Again, in an effort to avoid as much contamination as possible, since the work plan included amplifying reference samples (that would have a considerable amount of DNA), and also questioned samples (that would have few DNA), the setup of the experiment started by the latter and then the former, when performing the multiplex PCR and the capillary electrophoresis.

a) Calibration setup

When running the InnoTyper® 21 Human DNA Analysis Kit in an Applied Biosystems 3500 Genetic Analyser, there is a need to primarily run a Multi-capillary IGT 5 Dye Matrix Std. to spectrally calibrate the sequencer, in order to evaluate multicolour systems.

The IGT 5 Dye Matrix Std. consists in DNA fragments labelled with five different fluorescent dyes: FAM, JOE, TMR, ROX and TGI-ORANGE, used to perform the spectral calibration on the dye set G5. The generated file is important to be used during sampling detection in order to calculate the spectral overlap between the five dyes and separate the fluorescent signals in individual dye signals, using the size std. for IGT (ILS-155 internal lane Std.).

The matrix Std. was prepared considering the recommended quantities of each component for the 3500 sequencer, combining 9 µL of IGT 5 Dye Matrix Std. and 81 µL of Hi-Di™ Formamide (one capillary requires 1 µL of IGT 5 Dye and 9 µL of Hi-Di™ Formamide), being vortexed and centrifuged briefly. Then, 8 wells (one per each capillary) were filled with 10 µL each, being covered and heated at 95 ºC for 3 minutes (DNA denaturation). Consecutively, tubes were put on ice for 3 minutes and then were placed in Position A of the sequencer (A1 – H1 well positions). After the instrument preparation, the spectral calibration was run and checked for quality, being posteriorly saved.

b) Multiplex PCR

Firstly, using the TE^{-4} buffer, samples with great DNA amount were diluted to 0.3 ng (best previously known quantity, allowing to obtain complete genetic profiles with good quality). The RM was prepared (after having vortexed for 5 seconds the tubes) considering the recommended quantities of each component (Table 5), taking into account the 72 samples, plus 1 positive control, 1 NTC and some additional reactions to assure enough volume due to the loss that normally exists during the reagent transfers.

Table 5 - PCR multiplex Reaction Mix components and respective quantities per sample (InnoTyper® 21 Human DNA Analysis Kit).

PCR components for multiplex	Volume per Reaction 25 µL Total Reaction Mix
InnoTyper® Primer Mix	3.5 µL
InnoTyper® Master Mix	5.0 µL
IGT DNA Polymerase	0.5 µL
DNA Template/ Positive Control/ TE^{-4} buffer	Up to 16.0 µL

Initially, the RM was vortexed and briefly centrifuged, being 9 µL distributed in each of the 74 tubes. Posteriorly, obeying to a layout previously outlined, and using 8-tube PCR strips, 16 µL of TE^{-4} buffer were added to the NTC tube and then, 16 µL (or less) of questioned samples developed with cyanoacrylate were also added to their tubes. Sequentially, 16 µL (or less) of questioned samples without cyanoacrylate and 16 µL (or less) of reference samples were also added to the respective tubes, always pipetting up and down. When less than 16 µL of questioned or reference samples were added, TE^{-4} buffer was summed until the limit. At the end, the positive control was set up, adding 4 µL of the provided DNA control plus 12 µL of TE^{-4} buffer, pipetting up and down. All strips were capped, vortexed for 10 second and centrifuged at 3000 rpm for 20 seconds.

Obeying to a layout previously outlined, all 8-qPCR tube strips were introduced on the thermal cycler of the Bio-Rad CFX96 (Figure 17). A new protocol was created into the system with the specific cycling conditions for performing the specific run, that started immediately (Figure 23). At the end, samples were stored at -20°C.

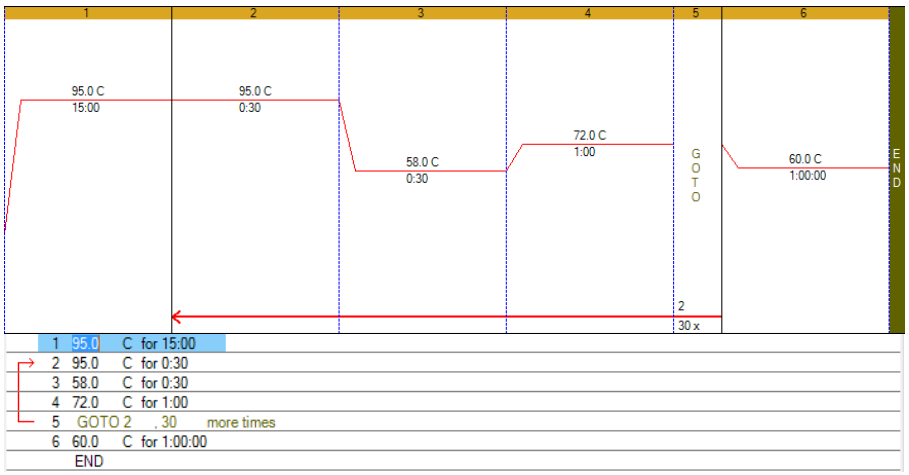


Figure 23 - Cycling Parameters for multiplex PCR of the InnoTyper® 21 Human DNA Analysis Kit.

c) Capillary electrophoresis – Fragment analysis

When performing fragment analysis, it is essential to have an allelic ladder running under the same conditions due to the possible different sizes that the same sample can present, in different instrument platforms (*e.g.* different polymers and/ or electrophoretic conditions). Thus, as recommended by the manufacturer, one allelic ladder was also run per each set of 23 samples. The instrument preparation preceded the samples run.

The RM was prepared considering the recommended quantities of each component (Table 6), taking into account the 72 samples, plus 1 positive control, 1 Non-template control (NTC), 4 allelic ladders, 2 blanks, and some additional reactions to assure enough volume due to the loss that normally exists during the reagent transfers

Table 6 - Capillary electrophoresis Reaction Mix components and respective quantities per sample (InnoTyper® 21 Human DNA Analysis Kit).

Reagent	Volume per Reaction
Hi-Di™ Formamide	10.6 µL
ILS-155 Internal Lane Standard	0.4 µL

Initially, obeying to a layout previously outlined, and using a 96-well sample plate, the 2 blank wells were set up having 11 µL of Hi-Di™ Formamide and 1 µL of IGT ILS-155 Internal Lane Std.. Although in the manufacturer's protocol it is written that blanks should have only 12 µL of Hi-Di™ Formamide, we choose to also add IGT ILS-155 Internal Lane Std. in order to be able to evaluate the peak sizes if Hi-Di™ Formamide was contaminated. Posteriorly, the RM was vortexed and briefly centrifuged, being 11 µL distributed in each of the 80 wells. Additionally, 1 µL of the negative control and 1 µL of questioned samples developed with cyanoacrylate were added to their wells. Sequentially, 1 µL of questioned samples without cyanoacrylate and 1 µL of reference samples were also added to the respective wells. At the end, 1 µL of the positive control was added to its well, as well as 1 µL of each allelic ladder, pipetting always up and down. The plate was sealed with an appropriate septum and briefly centrifuged. Moreover, the reaction plate was heated in a thermal cycler, for 3 minutes, at 95 °C and then immediately placed on ice for 3 minutes. Finally, the plate was placed on the autosampler tray into the instrument and the run started immediately.

Previously to data analysis, the import of InnoTyper® 21 panels and bins as well as the IGT ILS-155 Internal Lane Std. was ensured, and a new Casework Analysis Method was created.

IV. RESULTS AND DISCUSSION

4.1 VOLUNTEERS DATA

In accordance to the settings that were established previously to the recruitment of volunteers, the group encompassed 12 Caucasian donors (6 females and 6 males, with more than 18 years old (mean=27.4 years old; Table 7). Due to the framework of the current project, is it important to highlight the inexistence of the third phalange of the forefinger of the volunteer number 5.

Table 7 – Characterization of the volunteers of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing".

VOLUNTEERS	Age	Sex	Profession	HAND HYGIENE HABITS						Nail Biting	Sweating Self-evaluation	Steering Wheel Manipulation
				Nr. Washes/ day	Washing Products	Drying Material	Hand Antiseptics	Direção Geral de Saúde Handwashing Guidelines	Cream Application			
1	26	F	Student	4 - 6	Water & Soap	Towel	No	Yes	Sometimes	Sometimes	Normal	2 Hands Superior-middle
2	20	F	Student	1 - 3	Water & Soap	Towel	No	Sometimes	Yes	No	Almost non-existent	2 Hands Superior-middle
3	21	F	Student	1 - 3	Water & Soap	Towel	No	Sometimes	Sometimes	No	Normal	Left hand Superior-top
4	53	F	Unemployed	> 10	Water & Soap	Towel	Sometimes	Yes	Yes	Yes	Almost non-existent	2 Hands Superior-middle
5	29	F	Firefighter	4 - 6	Water & Soap	Towel	Sometimes	Sometimes	Sometimes	Yes	Almost non-existent	2 Hands Superior-middle
6	19	F	Student	4 - 6	Water & Soap	Paper	No	No	No	Sometimes	Normal	Right hand Centre-right
7	31	M	Firefighter	4 - 6	Water & Soap	Towel	Sometimes	No	Yes	No	Almost non-existent	Left hand Superior-top
8	19	M	Student	1 - 3	Only water	Paper	No	Sometimes	Sometimes	No	Normal	2 Hands Superior-middle
9	29	M	Firefighter	> 10	Water & Soap	Towel	Sometimes	Sometimes	No	Yes	Normal	2 Hands Superior-middle
10	38	M	Seller businessman	> 10	Water & Soap	Paper	Sometimes	Yes	Sometimes	Sometimes	Almost non-existent	Left hand Left side-middle
11	21	M	Student	4 - 6	Water & Soap	Towel	No	No	No	Yes	Normal	2 Hands Superior-middle
12	23	M	Student	4 - 6	Water & Soap	Towel	No	Yes	No	No	Normal	Left hand Superior-top

Globally, considering all volunteers, only 3 (25%) had professions that could probably cause some damage in the epidermal ridges (firefighters). Relatively to their hand hygiene habits, 50% washed their hands 4 to 6 times a day, being the other 50% equally splitted between 1 to 3 times or more than 10 times a day. Most of the volunteers washed hands using water and soap (solely 1 washed with only water), drying mostly with a towel (only 25% used paper). Additionally, the use of antiseptic products was not common (≅ 58% did not use and ≅ 42% used only sometimes) and the handwashing guidelines from the Direção Geral da Saúde were only always followed by ≅ 33% of the volunteers (25% did not use and ≅ 42% used sometimes). Only 25% of the volunteers always used cream, ≅ 42% used sometimes and ≅ 33% did not use. Moreover, nail biting was not common in the majority of the volunteers since ≅ 42% did not do it and ≅ 33% did it always (25% did it sometimes). Related to sweating, most of the volunteers (≅ 58%) considered having a normal state and some (≅ 42%) considered having almost none. Finally, considering the preferred hands position while driving, most of the volunteers used both hands, handling the superior part of the steering wheel, mainly in the middle (Table 7).

Considering all the female volunteers, only 1 (≅ 17%) had a profession that could probably cause some damage in the epidermal ridges (firefighter). Relatively to their hand hygiene habits, 50% washed their hands 4 to 6 times a day, ≅ 33% washed between 1 to 3 times and only one washed more than 10 times a day. All volunteers washed hands using water and soap, drying mostly with a towel (only 1 used paper). Additionally, the use of antiseptic products was not common (≅ 67% did not use and ≅ 33% used only sometimes) and the handwashing guidelines from the Direção Geral da Saúde were only always followed by ≅ 33% of the volunteers (1 did not use and 50% used sometimes). Only ≅ 33% of the volunteers always used cream, 50% used sometimes and one do not use. Moreover, around 50% had the habit of nail biting since ≅ 33% did it, ≅ 33% did not do it and ≅ 33% did it sometimes. Related to sweating, 50% of the volunteers considered having a normal state and the other 50% considered having almost none. Finally, considering the preferred hands position while driving, most of the volunteers used both hands, handling the superior part of the steering wheel, mainly in the middle (Table 7).

Finally, considering all the male volunteers, only 2 (≅ 33%) had professions that could probably cause some damage in the epidermal ridges (firefighters). Relatively to their hand hygiene habits, 50% washed their hands 4 to 6 times a day, ≅ 33% more than 10 times

and only 1 (≅ 17%) washed between 1 to 3 times a day. Most of the volunteers washed hands using water and soap (solely 1 washed with only water), drying mostly with a towel (only ≅ 33% used paper). Additionally, the use of antiseptic products was not common (50% did not use and 50% used only sometimes) and the handwashing guidelines from the Direção Geral da Saúde were only always followed by ≅ 33% of the volunteers (≅ 33% did not use and ≅ 33% used sometimes). Only one volunteer always used cream, ≅ 33% used sometimes and 50% did not use. Moreover, nail biting was not common in the majority of the volunteers since 50% did not do it, ≅ 33% did it always (1 did it sometimes. Related to sweating, most of the volunteers (≅ 67%) considered having a normal state and some (≅ 33%) considered having almost none. Finally, considering the preferred hands position while driving, 50% of the volunteers used both hands, handling the superior part of the steering wheel, mainly in the middle (Table 7).

4.2 MIMETIZATION OF A REAL-LIFE SCENARIO DRIVING A CAR

In order to be able to simulate a real life scenario driving a car, two equal Audi second hand steering wheels were bought and coated with proper skin (Figure 24) and a specific metal structure (Figure 25) was constructed to handle them spinning.

Figure 24 - Audi second hand steering wheels newly coated with skin.

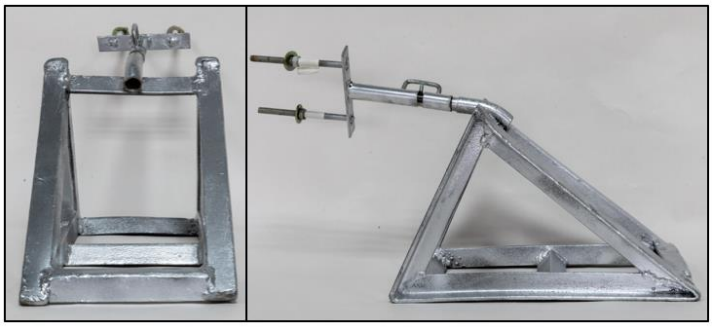


Figure 25 - Metal structure constructed to handle Audi steering wheels.

4.3 LOPHOSCOPY

4.3.1 LOPHOSCOPIC DATABASES

Although the present work has a reduced sample size to characterize the Portuguese population, the existence of population studies related to lophoscopic data is important in order to direct some possible investigations (*e.g.* identification of mass disaster victims), decreasing the range of possibilities.

4.3.1.1 FINGERPRINT DATABASE

After choosing the best 12 dactyloscopic bulletins for fingerprint analysis, 1 per each volunteer (*e.g.* Figure 26 and Figure 27), a population study was carried out considering, at the first moment, the dactyloscopic type and, at the second moment, the minutiae.

a) Dactyloscopic Type

Globally, considering all 12 volunteers, it was possible to verify that the "Monodéltico - Sinistrodéltico" type was the most predominant on 4 of the 5 fingers of the right hand (thumb and middle finger 75%; ring and little finger 83%). In the forefinger, the predominant dactyloscopic type was the "Bidéltico"/ "Polidéltico" (33%). Relatively to the 5 fingers of the left hand, the predominant dactyloscopic type was the "Monodéltico-Dextrodéltico" (thumb, ring finger and little finger 83%; forefinger 42%; middle finger 92%; (Table 8).

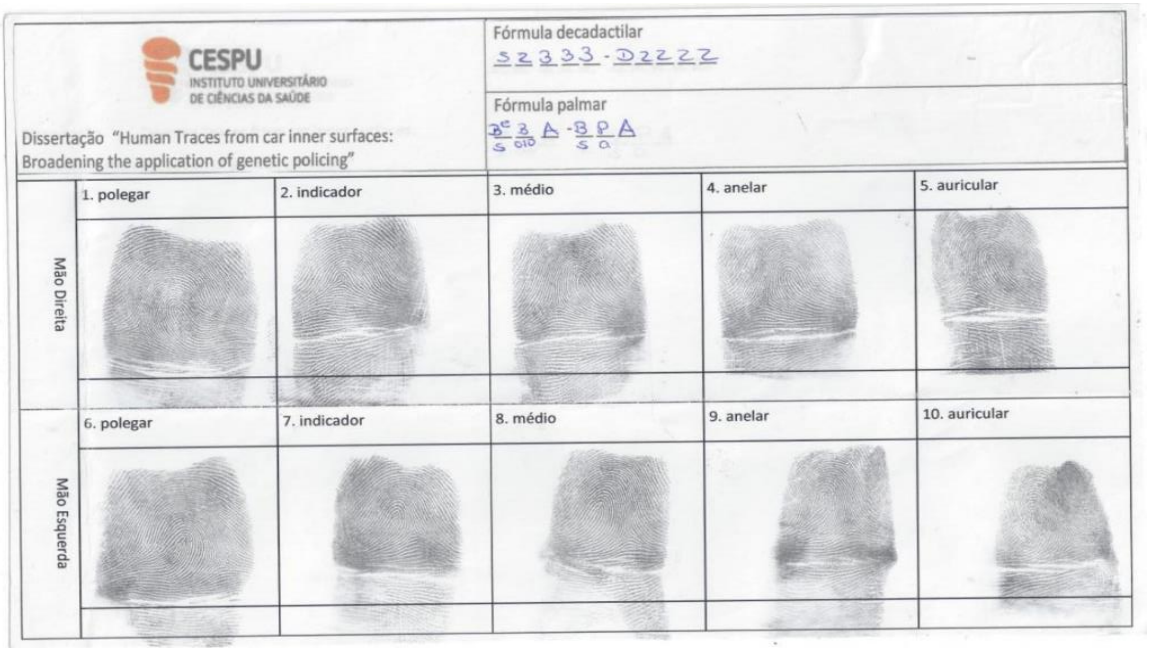


Figure 26 - Dactyloscopic bulletin of the volunteer K (front part).

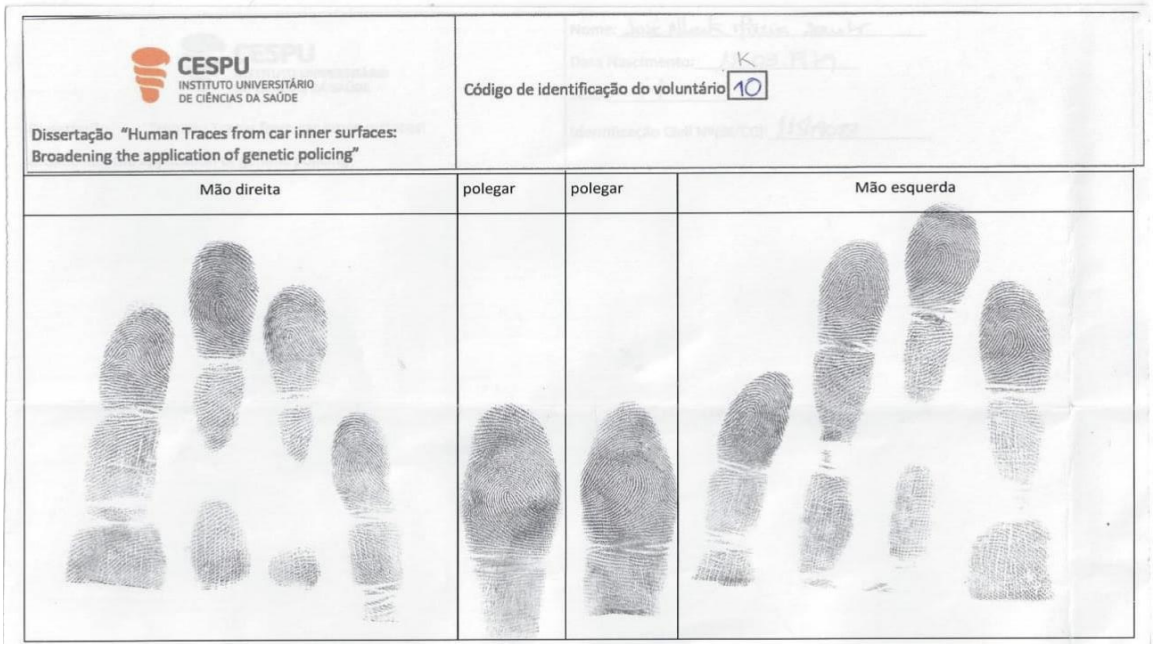


Figure 27 - Dactyloscopic bulletin of the volunteer K (reverse part).

Table 8 - Dactyloscopic types of the five fingers of the right hand and the five fingers of the left hand of the 12 volunteers (Olóriz System of Classification; *1 volunteer does not have the right forefinger; Bold=higher percentages).

		Dactyloscopic type % / Finger	Adéltico %	Monodéltico Dextrodéltico %	Monodéltico Sinistrodéltico %	Bidéltico Polidéltico %
All volunteers	Right Hand	Thumb	8	0	**75**	17
		Forefinger*	17	25	17	**33**
		Middle finger	8	17	**75**	0
		Ring finger	0	8,3(3)	**83.3(3)**	8.3(3)
		Little finger	0	8,3(3)	**83.3 (3)**	8.3(3)
	Left Hand	Thumb	17	**83**	0	0
		Forefinger	33	**42**	25	0
		Middle finger	8	**92**	0	0
		Ring finger	8.3(3)	**83.3(3)**	0	8.3(3)
		Little finger	8.3(3)	**83.3(3)**	8.3(3)	0

Considering all 6 female volunteers, it was possible to verify that the "Monodéltico-Sinistrodéltico" type was the most predominant of 4 of the 5 fingers on the right hand (thumb and ring finger 83%; middle and little finger 100%). In the forefinger, the predominant dactyloscopic type was the "Bidéltico" / "Polidéltico" (50%). Relatively to the 5 fingers of the left hand, the predominant dactyloscopic type was the "Monodéltico-Dextrodéltico" (thumb, middle, ring and little finger 100%; forefinger 67%; Table 9).

Table 9 - Dactyloscopic types of the five fingers of the right hand and the five fingers of the left hand of the 6 female volunteers (Olóriz System of Classification; *1 volunteer does not have the right forefinger; Bold=higher percentages).

		Dactyloscopic type % / Finger	Adéltico %	Monodéltico Dextrodéltico %	Monodéltico Sinistrodéltico %	Bidéltico Polidéltico %
Female volunteers	Right Hand	Thumb	0	0	**83**	17
		Forefinger*	0	17	17	**50**
		Middle finger	0	0	**100**	0
		Ring finger	0	0	**83**	17
		Little finger	0	0	**100**	0
	Left Hand	Thumb	0	**100**	0	0
		Forefinger	16.6(7)	**66.6(7)**	16.6(7)	0
		Middle finger	0	**100**	0	0
		Ring finger	0	**100**	0	0
		Little finger	0	**100**	0	0

Considering all 6 male volunteers, it was possible to verify that the "Monodéltico-Sinistrodéltico" type was the most predominant of four of the five fingers on the right hand (thumb and little finger 67%; middle finger 50%; ring finger 83%). In the forefinger, two dactyloscopic types were predominant the "Adéltico" (50%) and the "Monodéltico-Dextrodéltico" (50%). Relatively to the left hand, the "Monodéltico-Dextrodéltico" type was the most predominant of four of the five fingers (thumb, ring and little finger 67%; middle finger 83%). In the forefinger, the predominant dactyloscopic type was the "Adéltico" (50%; Table 10). Fingers from the male volunteers were more different from each other, when compared with fingers from the female volunteers.

Accordingly to the results obtained herein, a study carried out in the Portuguese population by Vilar (Vilar, 2015) also found that the "Monodéltico-Sinistrodéltico" was the predominant type for most of the fingers from the right hand and the "Monodéltico-Dextrodéltico" was the predominant type for most of the fingers from the left hand. The present study was also in accordance with the results obtained by Gutiérrez when studying a Spanish population (Gutiérrez-Redomero et al., 2011), relatively to the 2 most predominant dactyloscopic types of the right forefinger, being the "Monodéltico-Dextrodéltico" and "Bidéltico" / "Polidéltico".

Table 10 - Dactyloscopic types of the five fingers of the right hand and the five fingers of the left hand of the 6 male volunteers (Olóriz System of Classification; Bold=higher percentages).

		Dactyloscopic type % / Finger	Adéltico %	Monodéltico Dextrodéltico %	Monodéltico Sinistrodéltico %	Bidéltico Polidéltico %
Male volunteers	Right Hand	Thumb	16.6(7)	0	**66.7(7)**	16.6(7)
		Forefinger	**33**	**33**	17	17
		Middle finger	17	33	**50**	0
		Ring finger	0	17	**83**	0
		Little finger	0	16.6(7)	**66.7(7)**	16.6(7)
	Left Hand	Thumb	33	**67**	0	0
		Forefinger	**50**	17	33	0
		Middle finger	17	**83**	0	0
		Ring finger	16.6(7)	**66.7(7)**	0	16.6(7)
		Little finger	16.6(7)	**66.7(7)**	16.6(7)	0

b) Fingerprint minutiae

In Portugal, 14 main minutiae types are considered. However, in our sampling, there was no presence of "Pincel", "Secante", "Volta" and "Ramo".

Globally, considering all 12 volunteers, it was possible to verify that the most frequent fingerprint minutiae of the right and left hand were "Convergência" and "Bifurcação", followed by "Ponto" in the right hand and by "Fragmento" in the left hand, being the "Transversal" the less frequent. Relatively to both hands, the most frequent fingerprint minutiae were "Convergência" and "Bifurcação", followed by "Abrupta", being the "Tranversal" the less frequent (Table 11).

Table 11 - Mean values of the fingerprint minutiae of the five fingers of the right hand and the five fingers of the left hand of the 12 volunteers (*1 volunteer does not have the right forefinger; Bold=higher frequency; _=lower frequency).

		Right Hand						Left Hand						
	Fingerprint Minutiae	Thumb	Forefinger*	Middle finger	Ring finger	Little finger	Mean	Thumb	Forefinger	Middle finger	Ring finger	Little finger	Mean	Total mean
All volunteers	Abrupta	1.80	1.50	1.50	1.55	1.63	1.60	**1.58**	1.58	**1.40**	1.29	1.40	1.45	**1.52**
	Bifurcação	3.77	3.35	3.00	2.97	3.42	**3.30**	**3.67**	**3.08**	**3.50**	**3.92**	**3.00**	**3.43**	**3.37**
	Convergência	4.67	2.92	3.08	3.67	2.92	**3.45**	**3.67**	**4.08**	**4.00**	**3.42**	**3.55**	**3.74**	**3.60**
	Desviante	0.50	0.50	0.50	1.50	0	0.60	0.50	0.50	0.50	0	0	0.30	0.45
	Empalme	1.17	0.50	0.17	0.38	0.25	0.49	0.67	0.67	0.75	0.75	0.25	0.62	0.56
	Fragmento	1.58	1.80	1.17	1.33	1.50	1.48	1.50	1.33	0.67	**2.17**	**1.67**	**1.47**	1.47
	Interrupção	1.13	1.00	0.67	0.63	2.00	1.09	0.75	1.00	1.08	0.63	0.17	0.73	0.91
	Olhal	1.58	1.08	1.07	0.75	1.67	1.23	1.32	**1.75**	0.75	1.21	1.50	1.31	1.27
	Ponto	2.04	1.25	1.50	1.67	2.50	**1.79**	1.53	0	0.75	1.50	0.75	0.91	1.35
	Transversal	0.00	0.50	0.50	0	0	0.20	0	0.50	0	0	0	0.10	0.15

Considering all 6 female volunteers, it was possible to verify that the most frequent fingerprint minutiae of the right and left hand, and also considering both hand, were "Convergência", "Bifurcação" and "Fragmento", not existing the "Transversal" (Table 12).

Table 12 - Mean values of the fingerprint minutiae of the five fingers of the right hand and the five fingers of the left hand of the 6 female volunteers (*1 volunteer does not have the right forefinger; Bold=higher frequency; _=lower frequency).

		Right Hand						Left Hand						
	Fingerprint Minutiae	Thumb	Forefinger*	Middle finger	Ring finger	Little finger	Mean	Thumb	Forefinger	Middle finger	Ring finger	Little finger	Mean	Total mean
Female volunteers	Abrupta	0.60	1.00	0.83	0.60	1.25	0.86	1.00	1.00	0.80	1.25	1.00	1.01	0.93
	Bifurcação	3.20	3.20	2.00	2.60	3.00	**2.80**	2.83	2.83	3.00	2.50	2.83	**2.80**	**2.80**
	Convergência	4.17	3.20	2.83	3.00	2.67	**3.17**	3.17	3.17	2.83	2.33	2.60	**2.82**	**3.00**
	Desviante	1.00	1.00	1.00	3.00	0	1.20	0	1.00	0.00	0	0	0.20	0.70
	Empalme	2.33	1.00	0.33	0.75	0.50	0.98	1.33	1.33	1.00	1.50	0	1.03	1.01
	Fragmento	1.40	2.00	1.33	1.00	1.00	**1.35**	1.00	1.00	1.33	2.00	1.33	**1.33**	**1.34**
	Interrupção	1.25	1.00	1.33	1.25	1.00	1.17	1.50	0	0.50	1.25	0.33	0.72	0.94
	Olhal	1.50	1.00	0.80	0.50	1.00	0.96	0.83	1.50	1.00	1.25	1.50	1.22	1.09
	Ponto	1.33	0.50	1.00	1.00	0	0.77	1.25	0	0	0	0	0.25	0.51
	Transversal	0	0	0	0	0	0.00	0	0	0	0	0	0.00	0.00

Considering all 6 male volunteers, it was possible to verify that the most frequent fingerprint minutiae of the right and left hand were "Convergência" and "Bifurcação", followed by "Ponto" in the right hand and by "Abrupta" in the left hand, being the "Desviante" and "Empalme" the less frequent in the right hand and the "Empalme" and "Transversal" the less frequent in the left hand. Relatively to both hands, the most frequent fingerprint minutiae were "Convergência" and "Bifurcação" followed by "Ponto", being the "Empalme" the less frequent.

Table 13 - Mean values of the fingerprint minutiae of the five fingers of the right hand and the five fingers of the left hand of the 6 male volunteers (Bold=higher frequency; _=lower frequency).

		Right Hand						Left Hand						
	Fingerprint Minutiae	Thumb	Forefinger	Middle finger	Ring finger	Little finger	Mean	Thumb	Forefinger	Middle finger	Ring finger	Little finger	Mean	Total mean
Male volunteers	Abrupta	3.00	2.00	2.17	2.50	2.00	2.33	1.67	2.17	2.00	1.33	1.80	**1.79**	2.06
	Bifurcação	4.33	3.50	4.00	3.33	3.83	**3.80**	4.00	3.33	4.00	5.33	3.17	**3.97**	**3.88**
	Convergência	5.17	3.83	3.33	4.33	3.17	**3.97**	4.17	5.00	5.17	4.50	4.50	**4.67**	**4.32**
	Desviante	0	0	0	0	0	0.00	1	0	1.00	0	0	0.40	0.20
	Empalme	0	0	0	0	0	0.00	0	0	0.50	0	0.50	0.20	0.10
	Fragmento	1.75	1.60	1.00	1.67	2.00	1.60	2.00	1.67	0	2.33	2.00	1.60	1.49
	Interrupção	1.00	1.00	0	0	3.00	1.00	0	2.00	1.67	0	0	0.73	0.87
	Olhal	1.67	1.17	1.33	1.00	2.33	1.50	1.80	2.00	0.50	1.17	1.50	1.39	1.45
	Ponto	2.75	2.00	2.00	2.33	5.00	**2.82**	1.80	0	1.50	3.00	1.00	1.46	**2.14**
	Transversal	0	1.00	1.00	0	0	0.40	0	1.00	0	0	0	0.20	0.30

Results presented herein are different from the study carried out in the Portuguese population by Vilar (Vilar, 2015), that found the "Fragmento", "Olhal", and "Empalme" as the most frequent fingerprint minutiae. However, the results of the present work are similar with the ones obtained by Gutiérrez when studying a Spanish population (Gutiérrez-Redomero et al., 2011) , that found the "Abrupta", "Convergência" and "Bifurcação" as the most frequent fingerprint minutiae.

Despite the existence of few published population dactyloscopic studies and even with the limitation associated with the small number of volunteers in the current work (for this type of inference), it seems to exist a tendency for not being able to distinguish the Portuguese population from the Spanish population, by evaluating the dactyloscopic types and minutiae predominance.

4.3.1.2 PALMPRINT DATABASE

After choosing the best 12 quiroscopic bulletins for palmprint analysis, one per volunteer (*e.g.* Figure 28 and Figure 29), a population study was carried out considering the quiroscopic type.

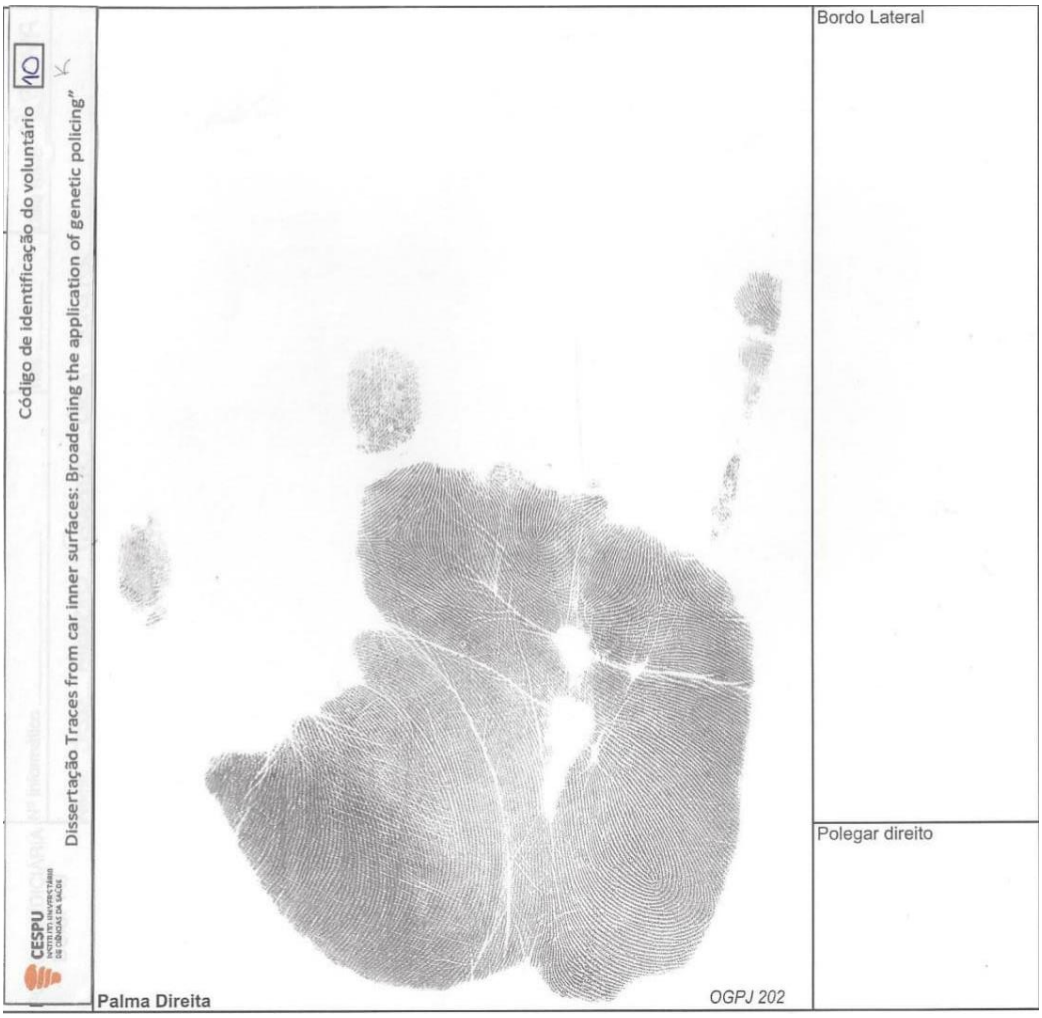


Figure 28 - Quiroscopic bulletin of the volunteer K (front part).

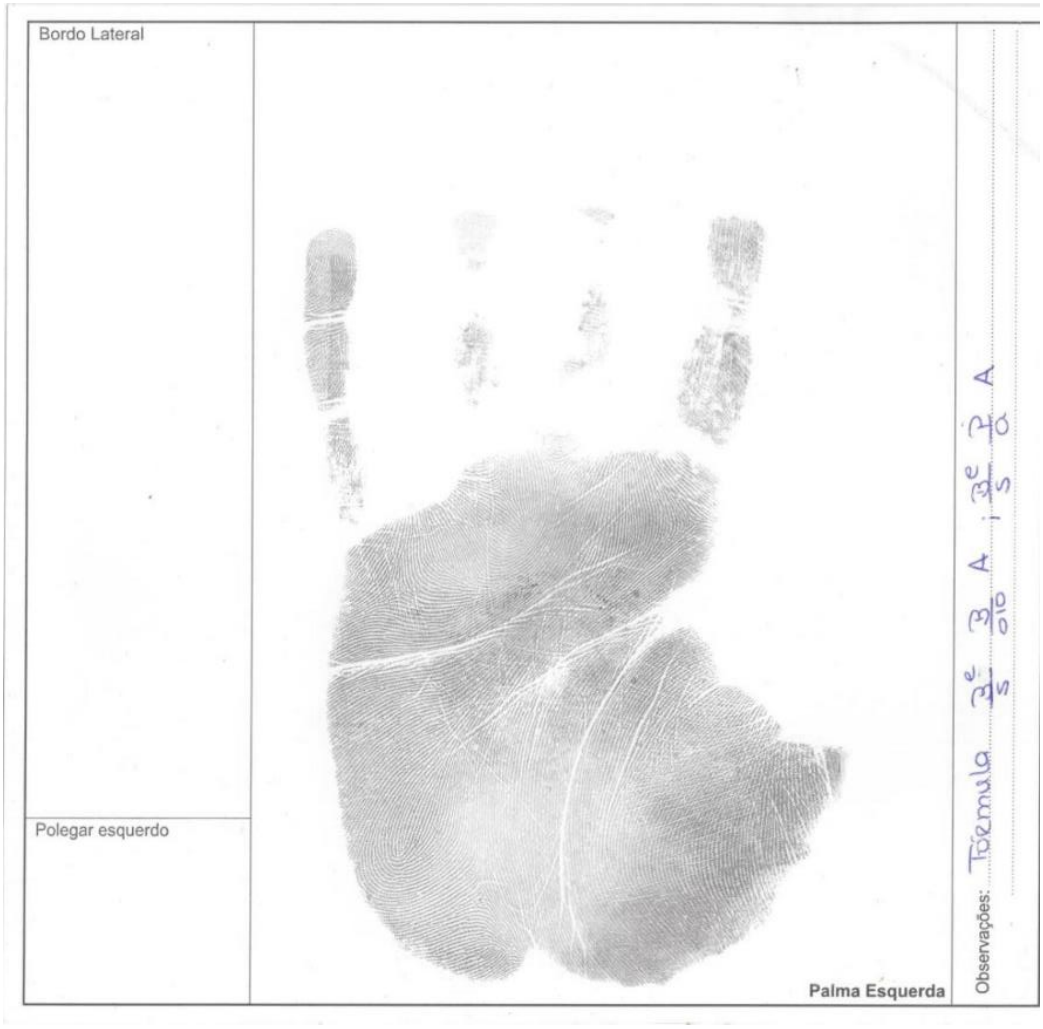


Figure 29 - Quiroscopic bulletin of the volunteer K (reverse part).

a) Quiroscopic Type

The hypothenar type "Verticilo" and the superior type "Misto" were not present in the sampling of the current study.

Globally, considering all 12 volunteers, it was possible to verify that the "Anucleado" type was the most predominant on 2 of the 3 regions of the right and left hand (hypothenar 67% and 50%, respectively; thenar 100% and 92%, respectively). In the superior region of the right and left hand, the predominant quiroscopic type was the "Bucleado" (92% and 67%, respectively; Table14Table 14).

Table 14 - Quiroscopic types of the three regions of the right hand and the three regions of the left hand of the 12 volunteers (Olóriz System of Classification; Bold=higher percentages; -=not present; N/A=not applicable).

		Types % / Regions	Anucleado %	Bucleado %	Duplo Bucle %	Piniforme %
All volunteers	Right Hand	Hypothenar	**67**	25	8	N/A
		Superior	-	**92**	N/A	8
		Thenar	**100**	-	-	N/A
	Left Hand	Hypothenar	**50**	33	17	N/A
		Superior	-	**67**	N/A	33
		Thenar	**92**	8	-	N/A

Considering all 6 female volunteers, it was possible to verify that the "Anucleado" type was the most predominant on 2 of the 3 regions of the right and left hand (hypothenar 50% and 33%, respectively; thenar 100% both). However, in the left hand there was an equal predominance (33%) of the 3 present types ("Anucleado", "Bucleado", "Duplo Bucle"). In the superior region of the right and left hand, the predominant quiroscopic type was the "Bucleado" (83% both; Table 15).

Table 15 - Quiroscopic types of the three regions of the right hand and the three regions of the left hand of the 6 female volunteers (Olóriz System of Classification; Bold=higher percentages; -=not present; N/A=not applicable).

		Types % / Regions	Anucleado %	Bucleado %	Duplo Bucle %	Piniforme %
Female volunteers	Right Hand	Hypothenar	50	33	17	N/A
		Superior	-	**83**	N/A	17
		Thenar	**100**	-	-	N/A
	Left Hand	Hypothenar	33.3(3)	33.3(3)	33.3(3)	N/A
		Superior	-	**83**	N/A	17
		Thenar	**100**	-	-	N/A

Considering all 6 male volunteers, it was possible to verify that the "Anucleado" type was the most predominant on 2 of the 3 regions of the right and left hand (hypothenar 83%

and 67%, respectively; thenar 100% and 83%, respectively). In the superior region of the right hand, the predominant quiroscopic type was the "Bucleado" (100%) and in the superior region of the left hand there was an equal predominance (50%) of the "Bucleado" and "Piniforme" (Table 16).

Table 16 - Quiroscopic types of the three regions of the and and the three regions of the left hand of the 6 male volunteers (Olóriz System of Classification; Bold=higher percentages; -=not present; N/A=not applicable).

Male volunteers		Types / Regions	Anucleado %	Bucleado %	Duplo bucle %	Pine %
	Right Hand	Hypothenar	83	17	-	N/A
		Superior	-	**100**	N/A	-
		Thenar	**100**	-	-	N/A
	Left hand	Hypothenar	67	33	-	N/A
		Superior	-	50	N/A	50
		Thenar	**83**	17	-	N/A

No quiroscopic population studies were found in order to be able to compare data.

4.3.2 LOPHOSCOPIC EXPERTISE

4.3.2.1 CYANOACRYLATE FUMING

All processed steering wheels presented deposition of cyanoacrylate on their surfaces. However, 2 presented just tenuous residues of cyanoacrylate without any kind of evidence (Figure 30 A, B), 6 presented tenuous, considerable or significant residues of cyanoacrylate, revealing traces with no identification value (Figure 30 C, D, E, G, I, J) and 4 presented a significant quantity of cyanoacrylate also having traces with an identification value (Figure 30 F, H , K, L).

Figure 30 - Driven steering wheels developed with cyanoacrylate fuming (A-L=volunteers codified according to the sampling sequence).

4.3.2.1 HITS AND CHARTINGS

Having already inserted into Automated Fingerprint Identification System (AFIS), the fingerprint and palmprint reference samples of the 12 volunteers, as well as the 4 questioned samples, the system was asked to find "Hits" between them. Due to the lack of an appropriate scale in 3 photographs, AFIS was not able to achieve the respective "Hits". Thus, knowing that a correspondence must exist, a Senior Forensic Expert evaluated the questioned and reference samples and established the 3 correspondences (*e.g.* Figure 31). Finally, the Expert confirmed the AFIS "Hit" and graphical demonstrations (chartings) of

each "Hit" were executed, revealing the existence of twelve equal and correspondent minutiae, also not having any natural dissimilarities (Figure 32, Figure 33, Figure 34, Figure 35). At the end, the Principal Investigator (PI) confirmed as correct, the Iophoscopic expertise.

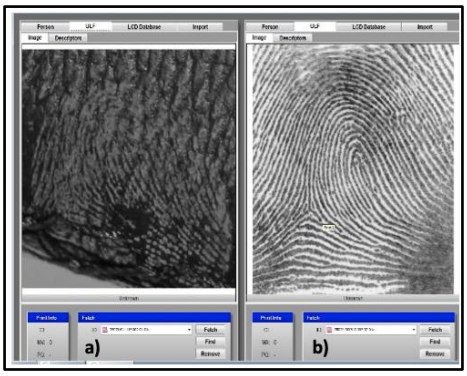


Figure 31 - Established "Hit" between the questioned fingerprint sample F (a)) and the reference fingerprint sample K (b)).

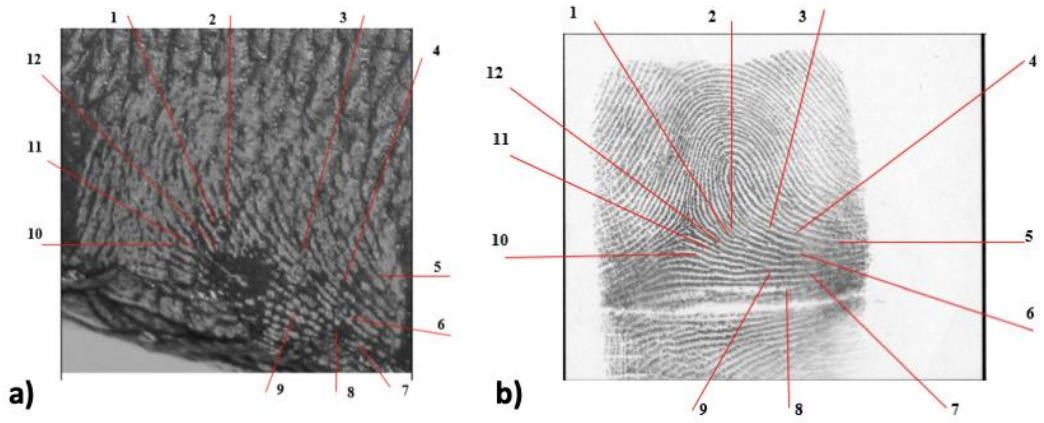


Figure 32 - Graphical demonstration of the established "Hit" between the questioned fingermark sample F (a)) and the reference fingerprint sample K (ring finger of the right hand; (b)).

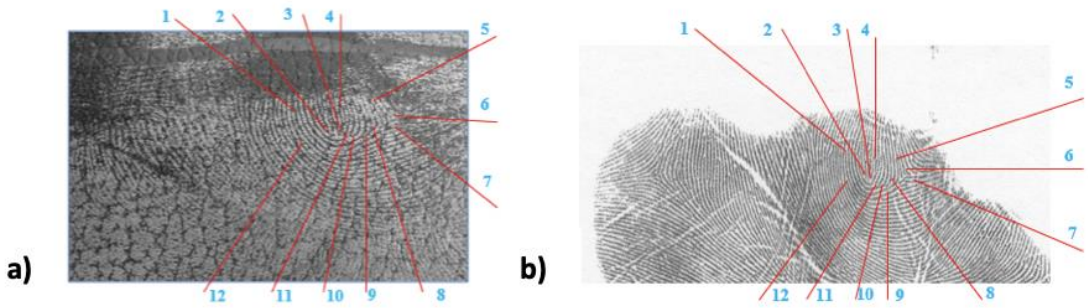


Figure 33 - Graphical demonstration of the established "Hit" between the questioned palm mark sample H (a)) and the reference palmprint sample C (superior region of the right hand (b))

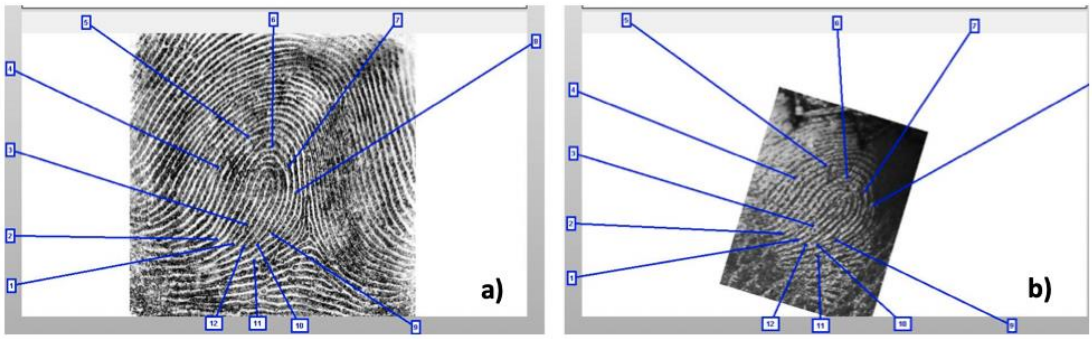


Figure 34 – Graphical demonstration of the established "Hit" between the reference fingerprint sample L (a) and the questioned fingermark sample K (middle finger of the left hand (b)).

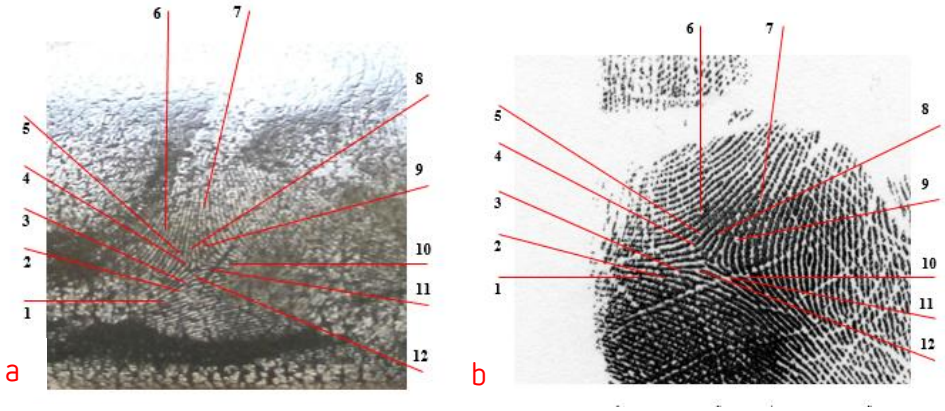


Figure 35 - Graphical demonstration of the established "Hit" between the questioned palmmark sample L (a)) and the reference palm print sample J (superior region of the right hand (b)).

In a universe of 12 volunteers, through lophoscopic expertise, it was possible to identify 33.3(3)% of the population that drove the steering wheels, in fact, more than it was expected based on the daily working life of the PJ lophoscopic experts. When trying to evaluate if there was a main side of the steering wheels where lophoscopic traces, with an identification value, appeared more frequently, it was seen that none correlation could be assumed since the distribution was similar around all the steering wheels, despite only few traces have been chosen for comparison. Thus, more time of hand contact with the left side of the steering wheel, did not reflect on a higher quality and quantity of the produced lophoscopic evidence.

Concerning to sex distribution, 50% of the identified drivers were females, being the other 50% males. Once more, no tendency could be observed. Deeping into the information of the collected questionnaires, knowing the identification number of each volunteer, it was possible to observe that all the identified drivers had professions that do not wear the papillary ridges (3 students and 1 seller businessman). Relatively to their hand hygiene habits, 50% washed hands 4 to 6 times a day, being the other 50% equally splitted between 1 to 3 times or more than 10 times a day. All the drivers washed hands using water and soap, drying mostly with a towel (1 used paper). Additionally, the use of antiseptic products was not common (1 used sometimes) and the handwashing guidelines from the Direção Geral da Saúde were only not followed by one driver. Related to cream application, 50% applied sometimes, being the other 50% equally splitted between applying or not applying. Moreover, the habit of nail biting and the self-evaluation related to sweating were equally distributed among the drivers (50 %/ 50 %). Finally, despite having the information about which were the habits related to steering wheel manipulation this feature was not explored since the driving simulation was standardized by imitating a video driver.

4.4 FORENSIC GENETICS (DATABASE AND EXPERTISE)

4.4.1 ASSESSMENT OF DNA QUANTIFICATION AND DEGRADATION

Normally, in forensic casework samples, it is required the DNA quantification priorly to the amplification of genomic DNA. InnoQuant® HY-R Kit is an extremely sensitive qPCR system specifically designed for forensic application, allowing not only to quantify total DNA but also to evaluate its degradation level. The system uses two independent autosomal retrotransposons (genomic targets; Figure 36): a multi-copy sequence of short length (80 bp; for quantification of total DNA) and a multi-copy sequence of long length (207 bp; for quantitative evaluation of DNA degradation state). The short target is a sequence from an *Alu* element, being the long target a sequence from an SVA element (Sine-R region), both having around 1800 copies per genome, allowing for high sensitivity and high reproducible quantification values (advantage over single-copy). The Degradation Index (DI) is perceived through the ratio between the short and long target, getting higher as long as the longer target is reduced due to their size susceptibility (Brown et al., 2017; Goecker et al., 2016;

Loftus et al., 2017) . A ratio equal to one means a high quality sample, while ratios above three mean moderate degradation (LeFebvre et al., 2017).

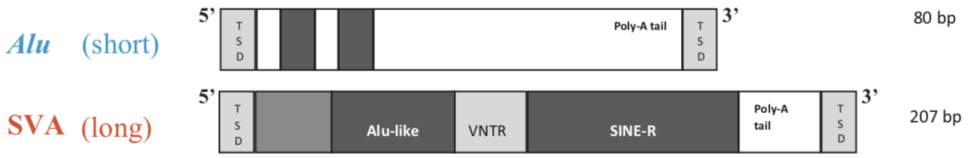


Figure 36 - Alu and SVA retrotransposons illustration (InnoQuant® HY-R Human and Male DNA Quantification & Degradation Assessment Kit (adapted from (Pineda et al., 2014)).

Additionally, the kit also allows to know, even during the quantification process, the sex of the person which DNA is being amplified since one of the amplification targets (80 bp) is located on the chromosome Y (genomic target: two multi-copy loci). Therefore, if the amplification of the chromosome Y target occurs the DNA came from a man and, if not, came from a woman, being this information also very important in cases of sexual assault when mixtures can be present. When studying DNA mixtures, the multi-copy Y targets allows to precisely quantify the male DNA, even when female DNA is highly concentrated (Loftus et al., 2017).

Lastly, the kit also uses a synthetic Internal Positive Control (IPC), included in the primer mix (172 bp), to assess a possible reaction inhibition by the evaluation of Cycle Threshold values (Auton et al., 2015) . The C_T value of a sample represents the number of the needed cycles to detect a fluorescent signal from the sample under analysis and at which a sample´s reaction curve intersects the threshold line, exceeding the background level. Therefore, C_T levels are inversely proportional to the quantity of the target in the sample (lower C_T value represents higher target amounts). The reaction inhibition can be evaluated since the ICT C_T value increases relatively to the C_T value of the standards (Std.), in the presence of Polymerase Chain Reaction (PCR) inhibitors (Loftus et al., 2017).

4.4.1.1 RUNNING QPCR PARAMETERS EVALUATION

Having finished the Real-Time PCR (qPCR) run, the system plate scheme was edited (Figure 37), in order to identify in which wells the samples were, also identifying which type of sample it was. Additionally, the concentration of each std. was specified and the amplification channels (fluorochromes) were selected, in accordance with the kit´s instructions (Figure 38).

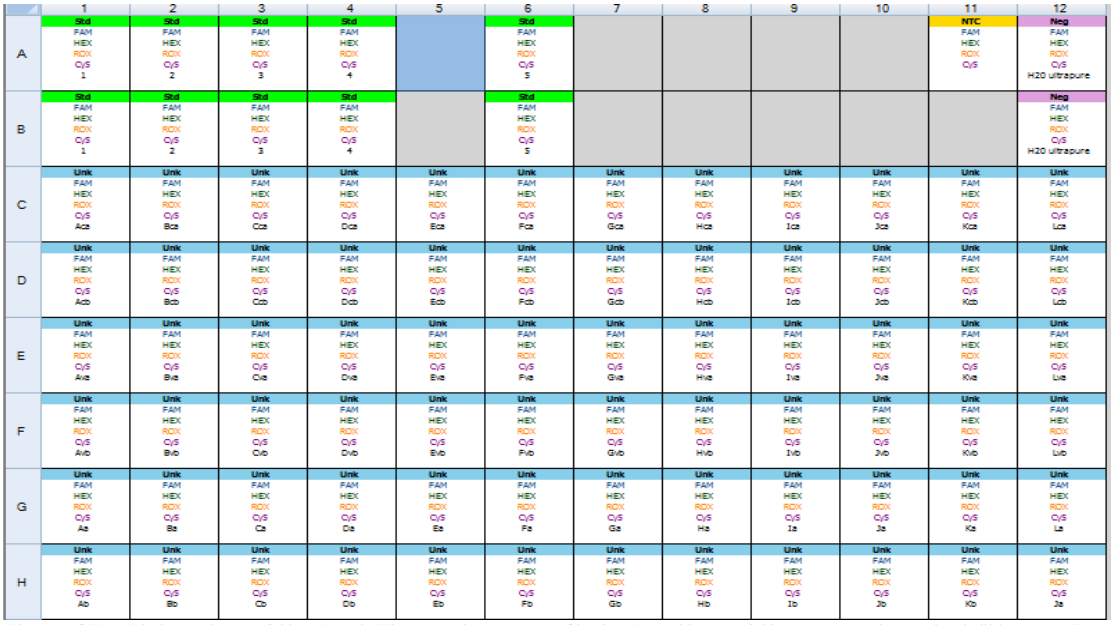


Figure 37 - Plate setup of the Real-Time Polymerase Chain Reaction of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing" (Std=standard; NTC=non template control; Unk=questioned samples; FAM HEX ROX, Cy5=amplification channels (fluorochromes)).

Short Target	FAM:	80 bp size
Long Target	Cy5:	207 bp size
Y Target	HEX:	80 bp size
Internal Positive Control (IPC)	ROX:	172 bp size

Figure 38 - DNA targets and respective amplification channels (fluorochromes; InnoQuant® HY-R Kit).

Posteriorly, the running parameters were evaluated in order to ensure that the run complied with the minimum requirements, so that, results could be validated and subsequently interpreted to be used. All requirements were fully fulfilled: efficiency values greater than 90% and less than 110% (short, long and Y targets; Figure 39); slope values between -3.6 and -3.1 (short, long and Y targets ; Figure 39); R^2 values greater than 0.98 (short, long and

Y targets; Figure 39); IPC C_T values no more than 2 units above the mean IPC C_T for all quantification Stds. on the plate (Figure 40).

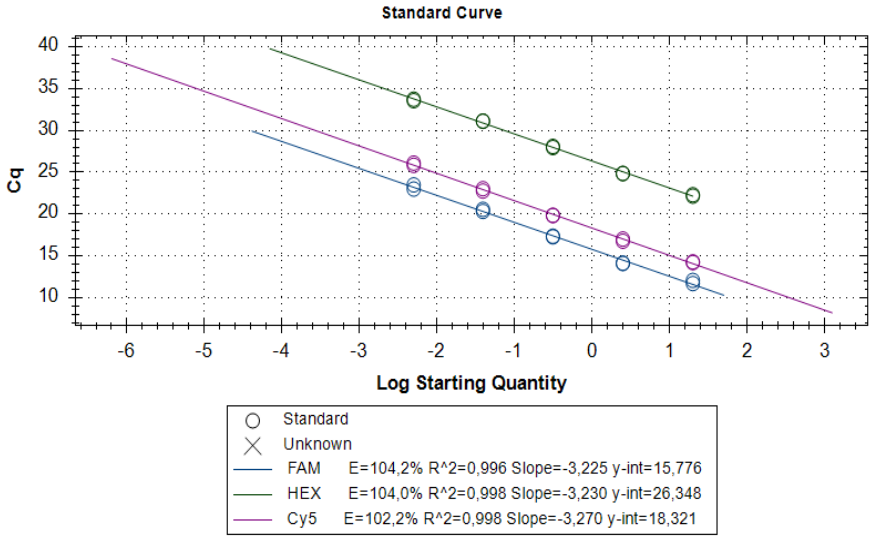
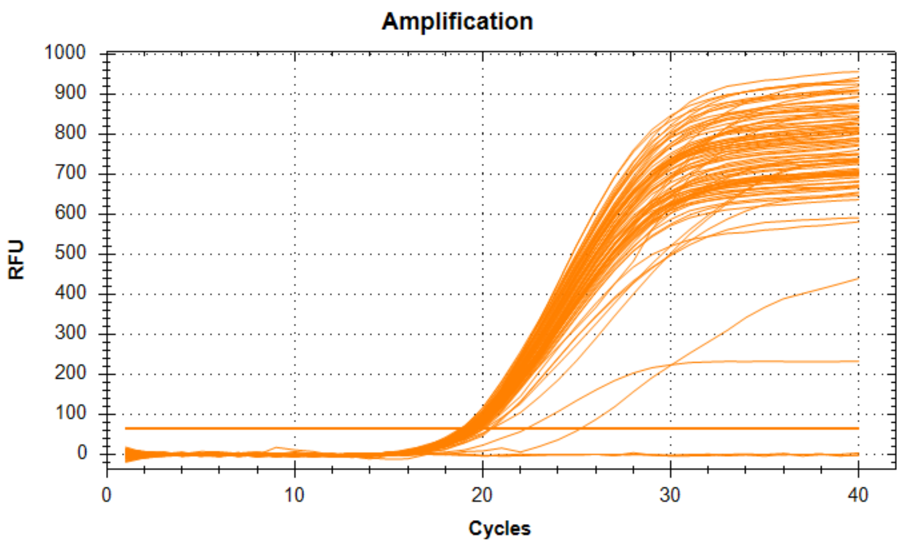


Figure 39 - Standard curves of the Real-Time Polymerase Chain Reaction of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing" (InnoQuant® HY-R Kit.

Since the mean of the C_T values of the IPC Stds. was 19.41, and C_T values higher than 21.41 represent the presence of PCR inhibitors, it was possible to observe that only two samples presented some reaction inhibition (*Lb*; *Ica*) and one sample did not present any amplification of the IPC target (*Gva*). In the reference sample *Lb*, an example of a possible inhibitor could be the excessive DNA concentration, however that was probably not the case since samples with higher concentration suffered IPC amplification (Figure 40, Figure 41). Related to the questioned sample *Ica*, cyanoacrylate could contribute to some inhibiton althought is should be also not that specificity since all of the other samples with cyanoacrylate amplified de ICT target.



		1	2	3	4	5	6	7	8	9	10	11	12
A	Content	Std	Std	Std	Std		Std					NTC	Neg Ctrl
	Sample	1	2	3	4		5						H20 ultrapure
	Cq	19,43	19,21	19,40	19,44		19,45					19,57	
	copy number	20,00	2,50	0,31	0,04		0,01						
B	Content	Std	Std	Std	Std		Std						Neg Ctrl
	Sample	1	2	3	4		5						H20 ultrapure
	Cq	19,38	19,34	19,48	19,55		19,46						
	copy number	20,00	2,50	0,31	0,04		0,01						
C	Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn
	Sample	Aca	Bca	Cca	Dca	Eca	Fca	Gca	Hca	Ica	Jca	Kca	Lca
	Cq	19,69	19,62	19,47	19,71	19,89	19,68	19,40	19,72	22,29	19,49	19,34	19,56
	copy number	0,00	0,00	0,01	0,00	0,00	0,00	0,94	0,00	0,00	0,00	30,91	0,00
D	Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn
	Sample	Acb	Bcb	Ccb	Dcb	Ecb	Fcb	Gcb	Hcb	Icb	Jcb	Kcb	Lcb
	Cq	19,45	19,63	20,34	19,53	19,42	19,56	19,45	19,65	20,03	19,80	19,38	19,61
	copy number	0,04	0,00	0,00	0,00	0,28	0,00	0,04	0,00	0,00	0,00	2,72	0,00
E	Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn
	Sample	Ava	Bva	Cva	Dva	Eva	Fva	Gva	Hva	Iva	Jva	Kva	Lva
	Cq	19,49	19,28	20,44	19,81	19,36	19,79		19,41	19,41	19,40	19,10	19,19
	copy number	0,00	1054,77	0,00	0,00	9,64	0,00		0,44	0,31	0,64	103423755,13	294606,42
F	Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn
	Sample	Avb	Bvb	Cvb	Dvb	Evb	Fvb	Gvb	Hvb	Ivb	Jvb	Kvb	Lvb
	Cq	20,03	19,38	19,27	19,17	19,29	19,83	19,16	19,32	19,34	19,15	19,59	19,16
	copy number	0,00	2,66	2052,98	1250096,06	632,85	0,00	2279745,37	106,77	25,10	4611133,26	0,00	1779241,15
G	Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn
	Sample	Aa	Ba	Ca	Da	Ea	Fa	Ga	Ha	Ia	Ja	Ka	La
	Cq	19,23	19,06	19,01	20,43	19,19	19,45	19,12	18,81	20,06	18,91	18,92	19,01
	copy number	20037,41	1068880686,26	16312586591,41	0,00	405212,69	0,03	24086718,91	3661841304461170,00	0,00	9669000862351,77	5699146639717,03	25356346177,08
H	Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn
	Sample	Ab	Bb	Cb	Db	Eb	Fb	Gb	Hb	Ib	Jb	Kb	Lb
	Cq	19,02	19,47	19,06	18,85	18,77	19,05	19,37	19,17	19,43	19,26	19,25	25,18
	copy number	9419233033,24	0,01	778758650,37	387298251672488,00	41108108456428900,00	1519877766,06	5,45	1029929,65	0,15	3079,27	8288,08	0,00

FAM | Cy5 | HEX | ROX | Run Information | +

Figure 40 - Internal Positive Control amplification of the Real-Time Polymerase Chain Reaction of the research project “Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing” (same colours meaning same volunteers; InnoQuant® HY-R Kit).

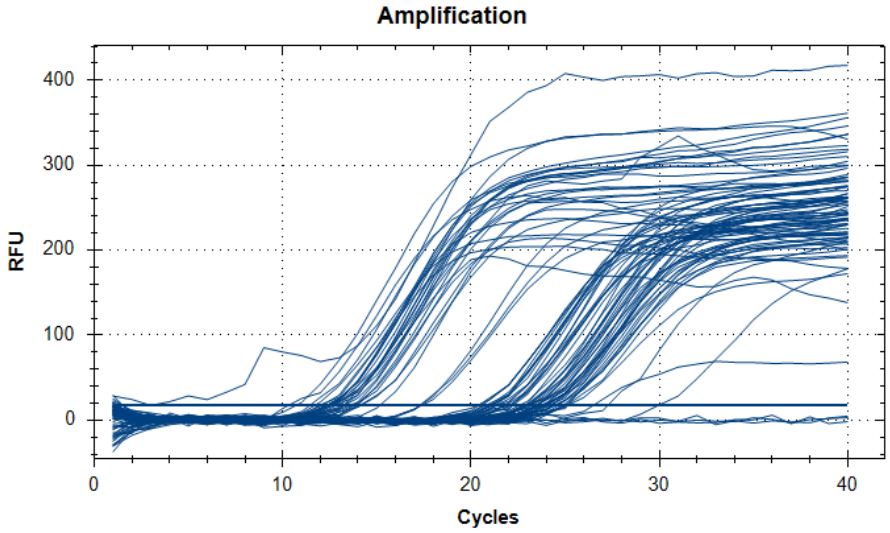
4.4.1.2 SAMPLE DNA QUANTIFICATION

The qPCR software produces calibration curves based on Stds., establishing the sample DNA concentration (Loftus et al., 2017). Having the running parameteres positively evaluated, the amplification of the short target was assessed in order to perceive the DNA concentration of each sample (Figure 41).

Since spurious signals resulting from ambient DNA or sporadic signal from the short or long targets (not so likely observed in the Y target due to the lower copy number) can be manifested, another manufacture´s advice is to disregard NTC or negative controls with no true amplification, meaning with C_T values higher than 30 (InnoGenomics Technologies, 2017) . The short target of the NCT of the current run had a C_T of 27.26. However, when observed the NCT C_T value of the long target (Figure 42), it was 30 and observing the NCT C_T value of the Y target (Figure 44) it was none, as it was expected. Due to that, and due to the fact that it is the highest C_T value among all samples (one exception, *Cca*), it is believed that even the NCT C_T value of the short target results from the referred neglectable influence.

With the DNA quantity data, in samples that had an excessive DNA concentration, it was possible to set the appropriate DNA input amount to amplify allowing for a more efficient workflow. It is the manufacture advise to dilute and re-quantify samples that have greater quantity than 20 ng/ µL (*Ga*, *Ia*, *Ib*) in order to assure an appropriate quantification value(Loftus et al., 2017), however this step was not performed due to the lack of kit´s reactions. In general, and as it was expected, the reference samples (*Aa-La* and *Ab-Lb*) revealed more DNA concentration when compared with all questioned samples. Into these, questioned samples without having applied any development agent (*Ava-Lva* and *Avb-Lvb*) revealed more DNA concentration when compared with the ones where cyanoacrylate was applied (*Aca-Lca* and *Acb-Lcb*). Probably cyanoacrylate had some negative influence not allowing for such a successful DNA extraction, thus concentration being smaller (Bille et al., 2009; von Wurmb et al., 2001). It is assumed that no influence occurred during the qPCR since among 24 samples, only one revealed some inhibition (Figure 41). Unexpectedly, was the inexistence of results in the sample *Jb* since no inhibition problem was revealed. However, when looking to its long target amplification (Figure 42), due to its high levels, it

is possible to suppose that probably the short target was so high that contributed for some inhibition.



		1	2	3	4	5	6	7	8	9	10	11	12		
A	Content	Std	Std	Std	Std		Std					NTC	Neg Ctrl		
	Sample	1	2	3	4		5						H2O		
	Cq	11,69	14,09	17,27	20,57		22,99					27,26			
	copy number	20,00	2,50	0,31	0,04		0,01								
B	Content	Std	Std	Std	Std		Std						Neg Ctrl		
	Sample	1	2	3	4		5						H2O		
	Cq	12,07	14,13	17,35	20,32		23,49								
	copy number	20,00	2,50	0,31	0,04		0,01							Mean	Mean
C	Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn		
	Sample	Aca	Bca	Cca	Dca	Eca	Fca	Gca	Hca	Ica	Jca	Kca	Lca		
	Cq	24,82	24,97	29,93	24,59	23,68	24,05	21,77	23,41	26,48	24,02	23,00	20,40	24,261	
	copy number	0,00157	0,00141	0,00004	0,00185	0,00353	0,00272	0,01381	0,00428	0,00048	0,00277	0,00575	0,03687	0,006	
D	Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn		
	Sample	Acb	Bcb	Ccb	Dcb	Ecb	Fcb	Gcb	Hcb	Icb	Jcb	Kcb	Lcb		
	Cq	24,27	24,24	24,15	25,13	23,40	23,86	22,04	21,35	25,11	23,86	21,35	20,92	23,310	23,785
	copy number	0,00232	0,00237	0,00253	0,00126	0,00432	0,00313	0,01113	0,01865	0,00126	0,00312	0,01864	0,02532	0,008	0,007
E	Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn		
	Sample	Ava	Bva	Cva	Dva	Eva	Fva	Gva	Hva	Iva	Jva	Kva	Lva		
	Cq	21,27	22,54	22,21	23,48	23,23	25,08		22,80	23,74	21,07	21,74	22,88	22,731	
	copy number	0,01976	0,00798	0,01009	0,00409	0,00490	0,00130		0,00665	0,00338	0,02286	0,01413	0,00629	0,009	
F	Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn		
	Sample	Avb	Bvb	Cvb	Dvb	Evb	Fvb	Gvb	Hvb	Ivb	Jvb	Kvb	Lvb		
	Cq	22,77	21,05	21,01	24,41	22,19	24,43	23,12	20,74	23,00	22,04	22,93	23,82	22,627	22,679
	copy number	0,00676	0,02308	0,02382	0,00210	0,01025	0,00207	0,00528	0,02889	0,00577	0,01144	0,00604	0,00321	0,011	0,010
G	Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn		
	Sample	Aa	Ba	Ca	Da	Ea	Fa	Ga	Ha	Ia	Ja	Ka	La		
	Cq	17,28	13,78	13,95	12,43	12,36	12,63	11,43	13,24	10,69	13,53	13,23	12,33	13,073	
	copy number	0,34	4,16	3,68	10,92	11,47	9,48	22,31	6,13	37,67	4,96	6,14	11,68	10,745	
H	Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn		
	Sample	Ab	Bb	Cb	Db	Eb	Fb	Gb	Hb	Ib	Jb	Kb	Lb		
	Cq	15,15	12,31	12,81	13,25	12,31	11,93	13,70	13,07	10,29		12,55	12,60	12,725	12,899
	copy number	1,57	11,90	8,29	6,08	11,89	15,61	4,40	6,89	50,20		9,98	9,63	12,403	11,574

FAM | Cy5 | HEX | ROX | Run Information | +

Figure 41 - Short target amplification of the Real-Time Polymerase Chain Reaction of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing" (same colours meaning same volunteers; InnoQuant® HY-R Kit).

On the other hand, samples *Lb* and *Ica*, that revealed some inhibition, had their short targets amplified as well as their long targets (Figure 42). As expected, *Gva* sample did not present any result since the same happened in the IPC target, being no amplification observed also in the long (Figure 42) and Y targets (Figure 44).

Although not being so discrepant, it is interesting to observe that the left part of the human body originated higher DNA quantity (Figure 41 mean red numbers). In the reference samples this pattern can possibly be related with eating habits, however, in the questioned samples this pattern can possibly be related to the time of contact with the steering wheel. Observing the video driving, the right hand has eleven minutes of contact with the steering wheel while the left hand has fourteen. Thus, since the left hand is during more time scrubbing the steering wheel, probably it releases a greater number of skin cells, resulting in higher DNA concentration.

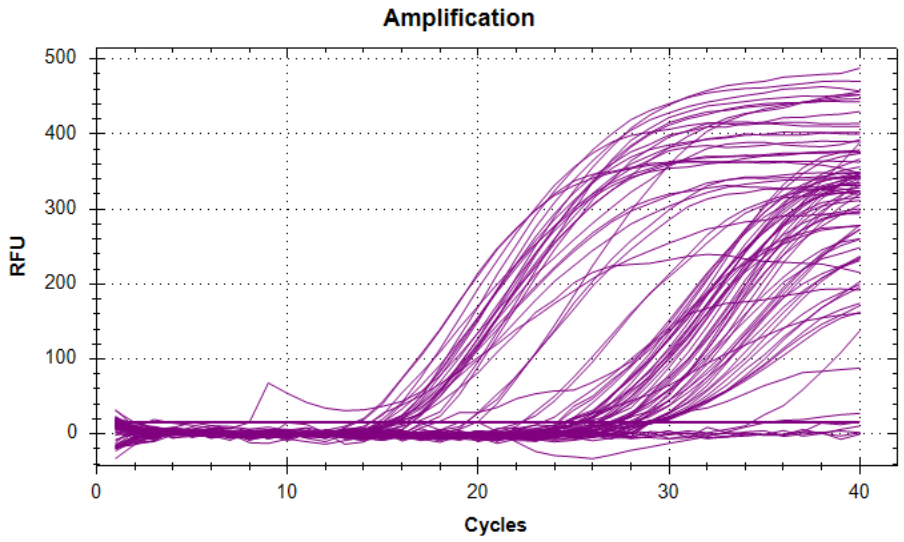
It was also possible to observe that, independently from the DNA source, females presented cyanoacrylate the mean DNA concentration for females was 0.011 ng/ µL, being for males 0.009 ng/ µL and, in samples developed with cyanoacrylate the mean DNA concentration for females was 0.009 ng/ µL, being for males 0.005 ng/ µL.

4.4.1.3 SAMPLE DEGRADATION INDEX

The amplification of the long target was also assessed (Figure 42), in order to obtain the DI of each sample, since it is the first target being attained due to its greater size.

Considering all samples of the current work, most of them revealed some degradation (Figure 43). It was possible to observe that reference samples had lower degradation indexes when compared with questioned samples. In reference samples most of the DI were between 1 and 3 while, in questioned samples, most of the degradation indexes were between 3 and 10. Thus, questioned samples, with low DNA quantity, presented higher degradation levels, being the DI correlated with the DNA quantity, confirming what is expected when dealing with forensic samples (Gouveia et al., 2017).

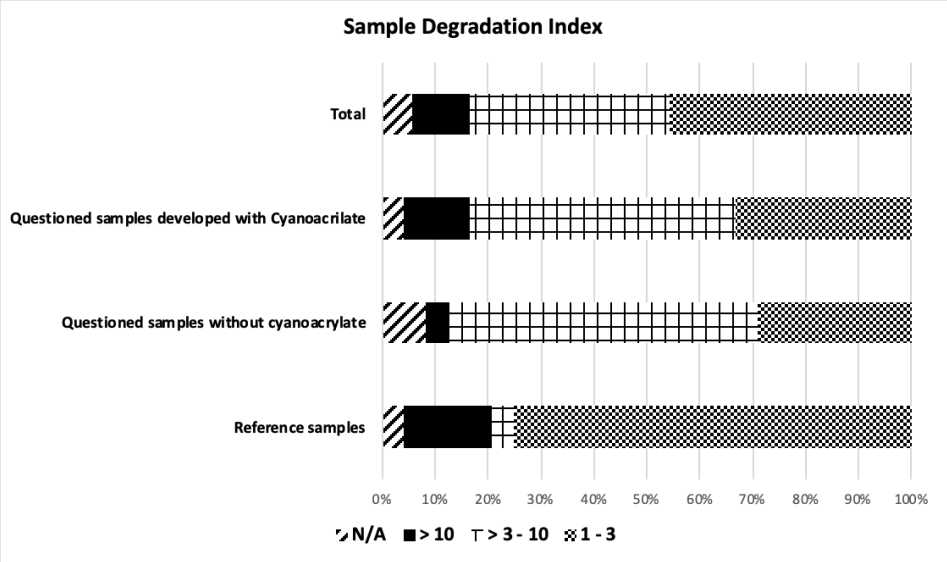
In 4 of the 72 samples it was not possible to measure the DI since they did not present measurements on their short target DNA (*Jb*), or Long target DNA (*Cva*; *Ccb*), or both (*Gva*).



		1	2	3	4	5	6	7	8	9	10	11	12		
A	Content	Std	Std	Std	Std		Std					NTC	Neg Ctrl		
	Sample	1	2	3	4		5						H2O		
	Cq	14,19	17,02	19,82	22,76		26,10					29,66			
	copy number	20,00	2,50	0,31	0,04		0,01								
B	Content	Std	Std	Std	Std		Std						Neg Ctrl		
	Sample	1	2	3	4		5						H2O		
	Cq	14,28	16,75	19,86	23,04		25,83							Mean	Mean
	copy number	20,00	2,50	0,31	0,04		0,01								
C	Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn		
	Sample	Aca	Bca	Cca	Dca	Eca	Fca	Gca	Hca	Ica	Jca	Kca	Lca		
	Cq	28,95	29,74	34,96	30,39	30,33	30,29	29,89	27,73	33,41	28,94	26,95	24,13	29,142	
	copy number	0,00056	0,00032	0,00001	0,00029	0,00021	0,00022	0,00042	0,00133	0,00009	0,00057	0,00230	0,01671	0,002	
D	Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn		
	Sample	Acb	Bcb	Ccb	Dcb	Ecb	Fcb	Gcb	Hcb	Icb	Jcb	Kcb	Lcb		
	Cq	28,83	29,84		29,72	28,03	30,38	28,27	25,83	30,37	28,00	25,32	24,53	27,921	28,531
	copy number	0,00061	0,00030		0,00033	0,00108	0,00020	0,00071	0,00504	0,00021	0,00109	0,00724	0,01264	0,003	0,003
E	Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn		
	Sample	Ava	Bva	Cva	Dva	Eva	Fva	Gva	Hva	Iva	Jva	Kva	Lva		
	Cq	25,60	26,83		29,14	26,68	30,11		27,12	27,72	26,23	26,30	27,91	27,365	
	copy number	0,00592	0,00250		0,00049	0,00278	0,00025		0,00204	0,00134	0,00380	0,00363	0,00117	0,002	
F	Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn		
	Sample	Avb	Bvb	Cvb	Dvb	Evb	Fvb	Gvb	Hvb	Ivb	Jvb	Kvb	Lvb		
	Cq	28,30	24,67	25,58	28,59	26,10	30,50	28,02	25,18	27,11	26,12	28,11	28,62	27,241	27,303
	copy number	0,00089	0,01143	0,00603	0,00072	0,00418	0,00019	0,00108	0,00799	0,00206	0,00413	0,00102	0,00071	0,003	0,003
G	Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn		
	Sample	Aa	Ba	Ca	Da	Ea	Fa	Ga	Ha	Ia	Ja	Ka	La		
	Cq	20,36	17,59	16,88	25,60	15,55	16,25	14,96	16,02	14,87	16,75	16,01	15,39	17,188	
	copy number	0,24	1,67	2,75	0,01	7,03	4,30	10,48	5,06	11,36	3,02	5,08	7,86	4,905	
H	Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn		
	Sample	Ab	Bb	Cb	Db	Eb	Fb	Gb	Hb	Ib	Jb	Kb	Lb		
	Cq	18,05	16,05	16,26	16,95	16,03	16,23	16,37	16,17	16,81	8,19	18,70	38,59	17,783	17,485
	copy number	1,21	4,96	4,27	2,63	5,00	4,33	3,94	4,55	2,89	1254,92	0,76	0,00	107,831	56,368

FAM | Cy5 | HEX | ROX | Run Information | +

Figure 42 - Long target amplification of the Real-Time Polymerase Chain Reaction of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing" (same colours meaning same volunteers; InnoQuant® HY-R Kit).



Sample	Std 1	Std 2	Std 3	Std 4		Std 5								
Short target	20,00	2,50	0,31	0,04		0,01								
Long target	20,00	2,50	0,31	0,04		0,01								
DI	1	1	1	1		1								
Sample	Std 1	Std 2	Std 3	Std 4		Std 5								
Short target	20,00	2,50	0,31	0,04		0,01								
Long target	20,00	2,50	0,31	0,04		0,01							Mean	Mean
DI	1	1	1	1		1								
Sample	Aca	Bca	Cca	Dca	Eca	Fca	Gca	Hca	Ica	Jca	Kca	Lca		
Short target	0,00157	0,00141	0,00004	0,00185	0,00353	0,00272	0,01361	0,00428	0,00439	0,00277	0,00575	0,03687	0,0	
Long target	0,00056	0,00032	0,00001	0,00035	0,00021	0,00022	0,00240	0,00133	0,00120	0,00057	0,00230	0,01671	0,0	
DI	2,81	4,38	5,02	9,04	16,56	12,41	5,71	3,23	2,39	4,91	2,51	2,21	3,0	
Sample	Acb	Bcb	Ccb	Dcb	Ecb	Fcb	Gcb	Hcb	Icb	Jcb	Kcb	Lcb		
Short target	0,00232	0,00237	0,00253	0,00126	0,00432	0,00313	0,01113	0,01865	0,00139	0,00312	0,01864	0,02532	0,0	0,0
Long target	0,00081	0,00030		0,00050	0,00106	0,00020	0,00371	0,00504	0,00037	0,00109	0,00724	0,01264	0,0	0,0
DI	3,81	7,90	N/A	3,84	4,01	15,25	3,00	3,70	6,19	2,85	2,58	2,00	2,7	2,8
Sample	Ava	Bva	Cva	Dva	Eva	Fva	Gva	Hva	Iva	Jva	Kva	Lva		
Short target	0,01976	0,00798	0,01009	0,00409	0,00490	0,00130		0,00665	0,00338	0,02286	0,01413	0,00629	0,0	
Long target	0,00592	0,00250		0,00049	0,00278	0,00025		0,00204	0,00134	0,00380	0,00363	0,00117	0,0	
DI	3,33	3,19	N/A	8,32	1,76	5,27	N/A	3,26	2,53	6,01	3,89	5,40	3,9	
Sample	Avb	Bvb	Cvb	Dvb	Evb	Fvb	Gvb	Hvb	Ivb	Jvb	Kvb	Lvb		
Short target	0,00676	0,02308	0,02382	0,00210	0,01025	0,00207	0,00528	0,02889	0,00577	0,01144	0,00604	0,00321	0,0	0,0
Long target	0,00089	0,01143	0,00603	0,00072	0,00418	0,00019	0,00108	0,00799	0,00206	0,00413	0,00102	0,00071	0,0	0,0
DI	7,60	2,02	3,95	2,89	2,45	10,98	4,89	3,62	2,80	2,77	5,94	4,54	3,2	3,5
Sample	Aa	Ba	Ca	Da	Ea	Fa	Ga	Ha	Ia	Ja	Ka	La		
Short target	0,34	4,16	3,68	10,92	11,47	9,48	22,31	6,13	37,67	4,96	6,14	11,68	10,7	
Long target	0,24	1,67	2,75	3,05	7,03	4,30	10,48	5,05	11,36	3,02	5,08	7,86	4,9	
DI	1,43	2,49	1,34	1837,59	1,63	2,20	2,13	1,21	3,32	1,64	1,21	1,49	2,2	
Sample	Ab	Bb	Cb	Db	Eb	Fb	Gb	Hb	Ib	Jb	Kb	Lb		
Short target	1,07	11,90	8,29	8,08	11,89	15,61	4,40	6,89	50,20		9,98	9,63	12,4	11,6
Long target	1,21	4,96	4,27	2,68	5,00	8,83	3,94	4,55	2,89	1254,92	0,76	0,00	107,8	56,4
DI	1,29	2,40	1,94	2,31	2,38	1,77	1,12	1,51	17,35	N/A	13,06	15183677,78	0,1	0,2

Figure 43 - Sample Degradation Index of the Real-Time Polymerase Chain Reaction of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing" (N/A=not available; same colours meaning same volunteers; InnoQuant® HY-R Kit).

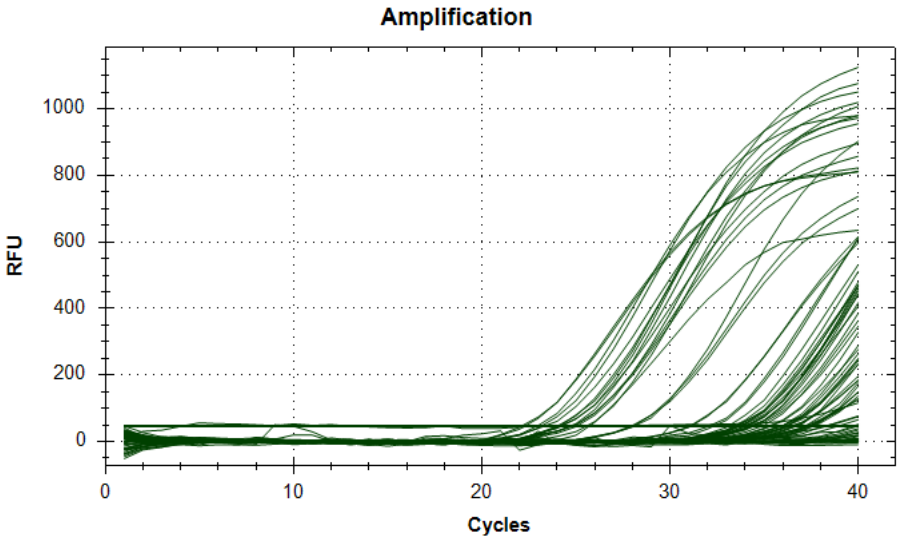
Observing the DNA concentration and DI of each sample, it is possible to speculate the expected results in relation to genetic profiling.

4.4.1.4 MALE DNA QUANTIFICATION

The InnoQuant® HY-R Y target is very important in the forensic arena, allowing the expert to know, from very earlier, if the questioned sample belongs to a male or female or even if the expert is dealing with a mixture of female and male DNA, when only female is expected (sexual assault).

In the present study, most of the male volunteers expressed their Y target (Figure 44). Exceptions were samples *Aca*, *Acb* and *Cca* where male target was not amplified. The inexistence of these amplifications could be associated with really low Y target quantities (Figure 41).

The main issue with the results presented herein (Figure 44) was the possible existence of male targets being amplified in female samples, seeming important to refer that experts involved in the practical work were female, not having any change of having contaminated the sample with their own DNA. When samples presented significant DNA quantities (24 reference samples), the existence of contamination being assumed as not possible, this event only occurred in one sample (Auton et al., 2015), with few Y target being amplified. In a retrospective analysis, it is known that this female volunteer came to be sampled together with her boyfriend. Thus, and since the cotton swab was sterile, a possible explanation could be the saliva exchange some minutes previously to the sampling. When getting into the questioned samples, that presented low DNA quantities, few were the female samples (5 in 48) that did not have the Y target being amplified. A possible explanation, since the steering wheels were only 2, could be the ineffectiveness of the cleaning method between volunteers.



		1	2	3	4	5	6	7	8	9	10	11	12
A	Content	Std	Std	Std	Std		Std					NTC	Neg Ctrl
	Sample	1	2	3	4		5						H2O
	Cq	22,14	24,87	27,95	31,13		33,56						
	copy number	20,00	2,50	0,31	0,04		0,01						
B	Content	Std	Std	Std	Std		Std						Neg Ctrl
	Sample	1	2	3	4		5						H2O
	Cq	22,32	24,84	28,09	31,09		33,74						
	copy number	20,00	2,50	0,31	0,04		0,01						
C	Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn
	Sample	Aca	Bca	Cca	Dca	Eca	Fca	Gca	Hca	Ica	Jca	Kca	Lca
	Cq		35,99		36,77	38,01	38,28	33,43	35,94	37,42	36,18	34,35	36,23
	copy number		0,0010		0,0008	0,0001	0,0002	0,0064	0,0011	0,0004	0,0009	0,0033	0,0009
D	Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn
	Sample	Acb	Bcb	Ccb	Dcb	Ecb	Fcb	Gcb	Hcb	Icb	Jcb	Kcb	Lcb
	Cq		36,23	36,58			36,00	33,13	37,45	36,47	34,93	34,19	37,22
	copy number		0,0009	0,0007			0,0010	0,0080	0,0004	0,0007	0,0022	0,0037	0,0004
E	Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn
	Sample	Ava	Bva	Cva	Dva	Eva	Fva	Gva	Hva	Iva	Jva	Kva	Lva
	Cq	23,56	33,72	39,78		34,53	38,36		34,04	34,45	34,33	37,36	37,21
	copy number	7,3048	0,0052	0,0001		0,0029	0,0002		0,0041	0,0031	0,0034	0,0004	0,0004
F	Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn
	Sample	Avb	Bvb	Cvb	Dvb	Evb	Fvb	Gvb	Hvb	Ivb	Jvb	Kvb	Lvb
	Cq		32,35	37,56		33,47	35,40	34,04	32,11	33,74	35,28	37,66	36,71
	copy number		0,0138	0,0003		0,0062	0,0016	0,0042	0,0165	0,0051	0,0017	0,0003	0,0006
G	Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn
	Sample	Aa	Ba	Ca	Da	Ea	Fa	Ga	Ha	Ia	Ja	Ka	La
	Cq	28,12	25,08	24,73			24,16				24,28		23,19
	copy number	0,28	2,47	3,18			4,76				4,38		9,52
H	Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn
	Sample	Ab	Bb	Cb	Db	Eb	Fb	Gb	Hb	Ib	Jb	Kb	Lb
	Cq	25,59	23,37	24,03			22,97				24,16	29,99	25,73
	copy number	1,72	8,35	5,22			11,12				4,76	0,07	1,55

FAM | Cy5 | HEX | ROX | Run

Figure 44 - Y Target amplification of the Real-Time Polymerase Chain Reaction of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing" (same colours meaning same volunteers; InnoQuant® HY-R Kit).

4.4.2 DNA TYPING

Regardless of the criminal or civil forensic context, genetic analysis ends in the establishment and comparison of genetic profiles. InnoTyper® 21 Human DNA Analysis Kit is an optimum typing system, specially designed for highly degraded DNA samples, being able to overcome forensic sample issues such as low DNA quantity and quality. It is believed that the success of applying this kit to analyse critical forensic samples is related to the very small and similar sizes of all multiplex PCR amplicons (63.4 – 123.5 bp; Table 17) (Sinha et al., 2015). This fact is due to the strategical design of the primers that overcomes the different sizes of the insertion and null alleles (differing between 200 to 400 bp) (Moura-Neto et al., 2018), also contributing to the lack of preferential amplification avoiding problems with the Peak Height Ratios (Auton et al.) and avoiding the existence of a greater number of allele dropouts (LaRue et al., 2012; Sinha, 2013; Sinha et al., 2015). Per each *Alu* marker, the primer design implies the existence of an equal forward primer for both alleles and two different reverse primers that assess the unique sequences of the *Alu* insertions in different genomic positions (Figure 45). One reverse primmer anneals only in the absence of the *Alu* element and the other reverse primer anneals to a sequence recognizing the named Target Site Duplication (TSD), a direct repeat sequence at the beginning and at the end of each *Alu* element. Both alleles are amplified in the presence of a heterozygous individual for a specific marker. With this primer strategy, some amplicons of the same marker can only differ 1 pb (Brown et al., 2017).

Table 17 - Information of the DNA markers of the InnoTyper® 21 Human DNA Analysis Kit (amplicon sizes measured in base pairs)

	Marker	Dye	Chromo-some	Band	Amplicon Size* (I = Insertion)	Amplicon Size* (N = No Insertion)	Positive Control Profile
1	AC4027	FAM	7	7q21.11	66.1	68.4	I,I
2	MLS26	FAM	3	3p22.1	79.8	82.3	I,N
3	ALU79712	FAM	20	20p12.2	91.6	95.4	N,N
4	NBC216	FAM	7	7p14.1	99.8	109.2	I,I
5	NBC106	FAM	21	21q22.2	115.9	120.0	I,N
6	RG148	JOE	2	2q23.3	73.3	80.5	I,N
7	NBC13	JOE	16	16p12.1	85.4	89.4	N,N
8	AC2265	JOE	13	13q33.1	96.4	100.5	I,N
9	MLS09	JOE	1	1q25.3	111.6	116.7	I,N
10	AC1141	TMR	3	3q11.2	63.4	66.2	I,I
11	TARBP	TMR	1	1q42.2	70.3	73.7	I,N
12	AMEL	TMR	X, Y	Xp22.1-22.3 Yp11.2	X=78.1	Y=80.9	X,Y
13	AC2305	TMR	13	13q13.3	92.0	97.2	I,N
14	HS4.69	TMR	5	5q34	109.2	113.9	I,N
15	NBC51	TMR	3	3q28	123.5	119.7	N,N
16	ACA1766	ROX	8	8q12.1	69.5	75.5	I,N
17	NBC120	ROX	22	22q11.21	79.2	83.6	I,N
18	NBC10	ROX	4	4q31.21	86.9	93.1	N,N
19	NBC102	ROX	17	17q23.3	101.0	97.1	N,N
20	SB19.12	ROX	19	19q13.43	108.7	113.6	I,N
21	NBC148	ROX	14	14q31.1	117.5	120.2	I,I

*** Observed sizes on 3130 Genetic Analyzer**

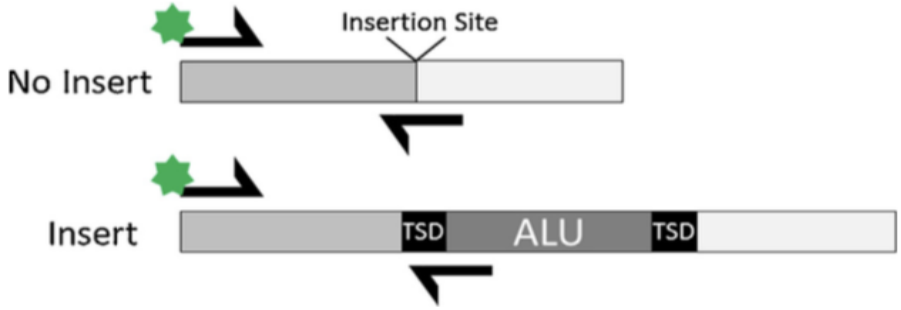


Figure 45 - Primer design strategy of *Alu* markers of the InnoTyper® 21 Human DNA Analysis Kit (TSD=target site duplications; InnoGenomics Technologies (adapted from (Brown et al., 2017).

The system allows to obtain complete genetic profiles with an input of 0.05 ng of DNA template and, with only 0.025 ng, partial profiles can still be obtained. Complete fragment analysis includes the amplification of 20 *Alu* dimorphic (INNUL) markers that are present in most of the populations, and the amplification of the sex determining marker Amelogenin (Brown et al., 2017). Therefore, InnoTyper® 21 Human DNA Analysis Kit is more discriminating when compared with the sequencing of the mitochondrial DNA which is the

most used alternative to analyse degraded forensic samples. This novel multiplex DNA analysis can be an important complement to the STR Kits that can still be used when samples are not so critical (*e.g.* paternity testing).

4.4.2.1 MULTIPLEX PCR

The experiment that was performed before running the samples of the current work was crucial to decide the best DNA quantity to input for multiplex PCR. Thus, it was decided that, when possible, DNA input would be 0.3 ng (advised quantity between 0.2 – 0.5 ng). Posteriorly, knowing the concentration of all samples under analysis, all reference samples were diluted to the whished DNA quantity (Table 18). Since the reference sample *Jb* did not have its short target amplified, is was not possible to set up the aimed DNA input. Thus, to overcome this issue and assure the obtention of two genetic profiles of the same donor, 0.3 ng DNA of the sample *Ja* (despite not being from the same cheek) were used. On the other hand, most of the questioned samples had really low DNA concentrations, being not possible, in these cases, to set up the 0.3 ng DNA quantity. Related to this fact, since it was not possible to concentrate samples due to the lack of the kit´s reactions, the maximum volume of each sample (16 µL) was input for multiplex PCR, being obtained specific quantities per each sample (Table 18). However, few questioned samples (*Gca*; *Hcb*; *Kcb*; *Lca*; *Lcb*; *Ava*; *Bvb*; *Cvb*; *Hvb*; *Jva*; *Kva*) had considerable DNA concentrations thus, they were set up to only 0.2 ng DNA (avoiding a bigger concentration discrepancy with other questioned samples).

At this point, based on the minimal needed input to obtain complete and partial genetic profiles (InnoGenomics Technologies, 2017), it was expected to have complete profiles of all reference samples (not critical samples). Related to the global questioned samples is was expected to have ≅ 66.7% of complete profiles, ≅ 20.8% of partial profiles and 12.5% of none profiles. Specifying, into the questioned samples without cyanoacrylate it was expected to have ≅ 83.3% of complete profiles, 12.5% of partial profiles and ≅ 4.2% of none profiles. Finally, into the questioned samples developed with cyanoacrylate it was expected to have 50% of complete profiles, ≅ 29.2% of partial profiles and ≅ 20.8% of none profiles (Table 18).

Table 18 - Amount of DNA input for Multiplex Polymerase Chain Reaction of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing" (ng=nanograms; yellow samples=partial expected profiles; green samples=none expected profiles; blue samples=complete expected profiles)

Samples	DNA input (ng)	Samples	DNA input (ng)	Samples	DNA input (ng)
Aca	0.0251	Ava	0.2	Aa	0.3
Acb	0.0372	Avb	0.1081	Ab	
Bca	0.0226	Bva	0.1277	Ba	
Bcb	0.038	Bvb	0.2	Bb	
Cca	0.0007	Cva	0.1614	Ca	
Ccb	0.0405	Cvb	0.2	Cb	
Dca	0.0296	Dva	0.0655	Da	
Dcb	0.0201	Dvb	0.0336	Db	
Eca	0.0565	Eva	0.0784	Ea	
Ecb	0.0691	Evb	0.1639	Eb	
Fca	0.0436	Fva	0.0209	Fa	
Fcb	0.05	Fvb	0.0332	Fb	
Gca	0.2	Gva (Gvb)	≅ 0.0264	Ga	
Gcb	0.1781	Gvb	0.0845	Gb	
Hca	0.0685	Hva	0.1064	Ha	
Hcb	0.2	Hvb	0.2	Hb	
Ica	0.0077	Iva	0.0541	Ia	
Icb	0.0204	Ivb	0.0923	Ib	
Jca	0.0444	Jva	0.2	Ja	
Jcb	0.05	Jvb	0.183	Jb (Ja)	
Kca	0.092	Kva	0.2	Ka	
Kcb	0.2	Kvb	0.0966	Kb	
Lca	0.2	Lva	0.1007	La	
Lcb	0.2	Lvb	0.0514	Lb	

4.4.2.2 CAPILLARY ELETROPHORESIS – FRAGMENT ANALYSIS

The InnoTyper® 21 Human DNA Analysis Kit uses as Internal Lane Standard, the IGT ILS-155 (Figure 46), composed of 11 fragments (55, 60, 70, 85, 95, 105, 115, 125, 135, 145, 155 bp) which allow measuring the height of the obtained peaks.

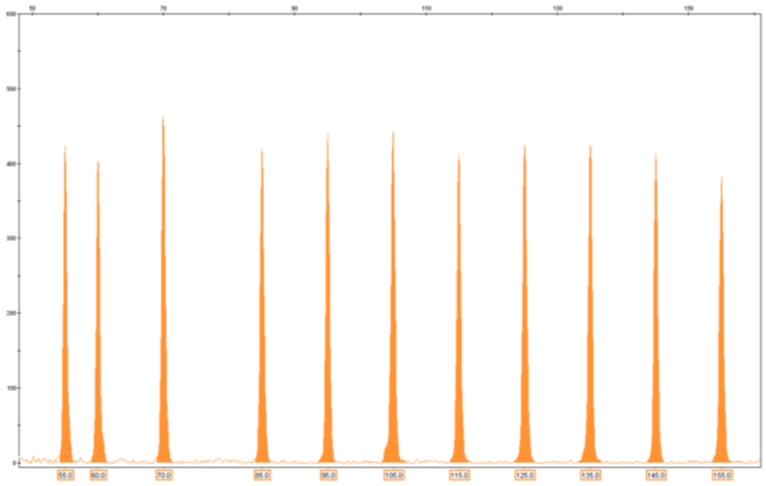


Figure 46 - Internal Lane Standard (IGT ILS-155) of the InnoTyper® 21 Human DNA Analysis Kit (addapted from (InnoGenomics Technologies, 2017)).

The system also uses an allelic ladder (Figure 47), that have the common alleles that are present in the human population for each particular DNA marker (total of 40 different INNUL alleles and Amelogenin X and Y). The allelic ladder is used as a reference DNA size for each allele, being important for correct genotype determinations. Peaks labelled with "I" represent the insertion alleles and peaks labelled with "N" represent the null alleles.

Previously to perform the genotype determinations of the reference and questioned samples, based on their genetic profiles, controls (4 Allelic Ladders; 1 negative control and 1 positive control), were evaluated in order to observe if they had produced the expected results (Figure 48, Figure 49, Figure 50). As it was observed, allelic ladders presented the expected results only varing relatively to peak sizes (*e.g.* Figure 48). Variation in peak sizes are within the expected due to the use of different instruments and running conditions. Related to the negative control, results were also in accordance to the expected. However, it was possible to observe few artefacts mainly in the blue (1 artefact around 92 bp) and green (1 artefact around 90 bp) dye channels. When using Pop 7, both artefacts were already reported (Lowther et al., 2016), being this issue probably associated with the use of a different polymer and capillary array (Pop 4 and 36 cm capillary arrays are recommended by the manufacturer). Finally, when comparing the genotype of the positive control (Table 17) and the obtained genetic profile (Figure 50), results were also similar to the expected.

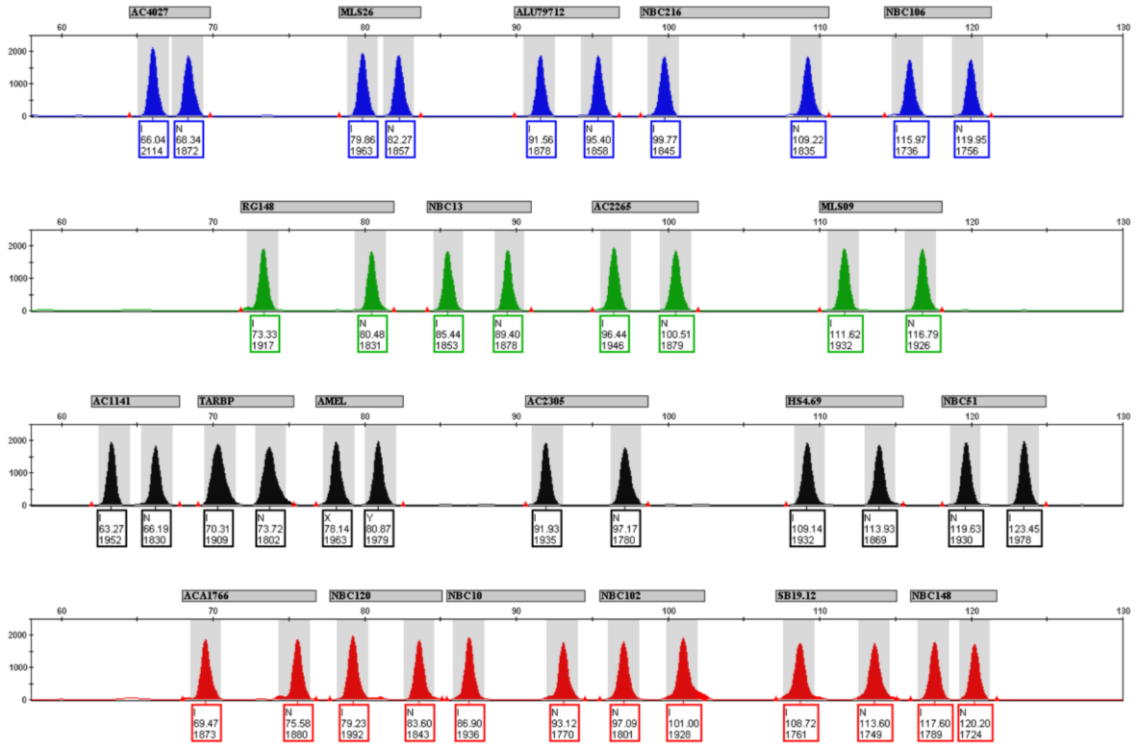


Figure 47 - Allelic Ladder information of the InnoTyper® 21 Human DNA Analysis Kit (addapted from (InnoGenomics Technologies, 2017)).

However, since the artefact of the blue chanel set on the insertion allele of the ALU79712 marker (non-existent in this positive control), some interference occurred assuming the individual as homozygous for the presence of the insertion and the true alleles (N homozygous) were not read, thus, they had to be manually added. Additionally, the artefact of the green channel set on the null allele of the NBC13 marker. As the positive control is homozygous to this allele, the only problem that appeared was an abnormal peak size/ area. Problems in these two markers appeared in most of the genetic profiles so, these markers were not significantly considered when trying to find a match between questioned and reference samples.

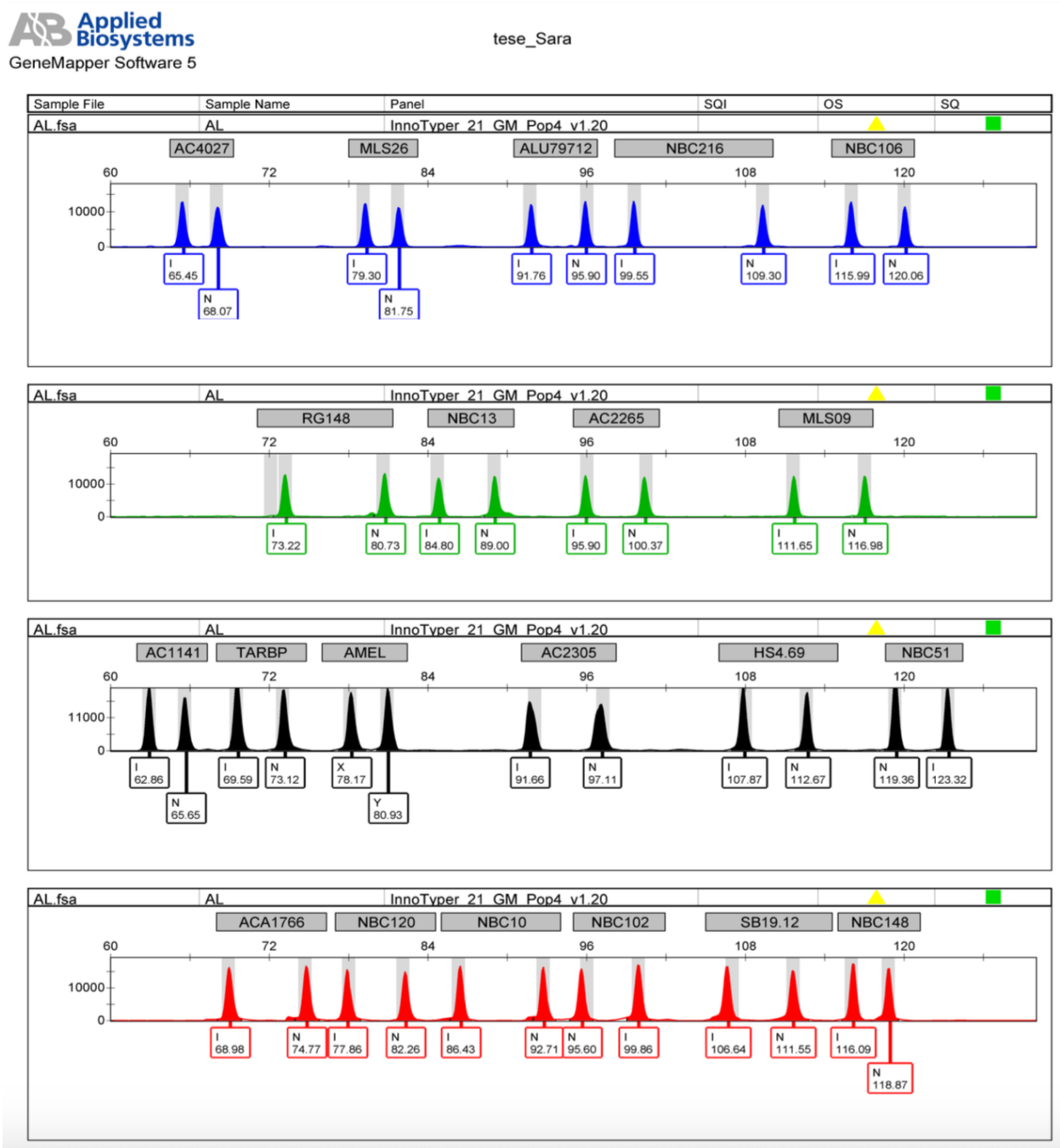


Figure 48 - Allelic Ladder information (markers, alleles and peak sizes) from the capillary electrophoresis of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing" (InnoTyper® 21 Human DNA Analysis Kit).

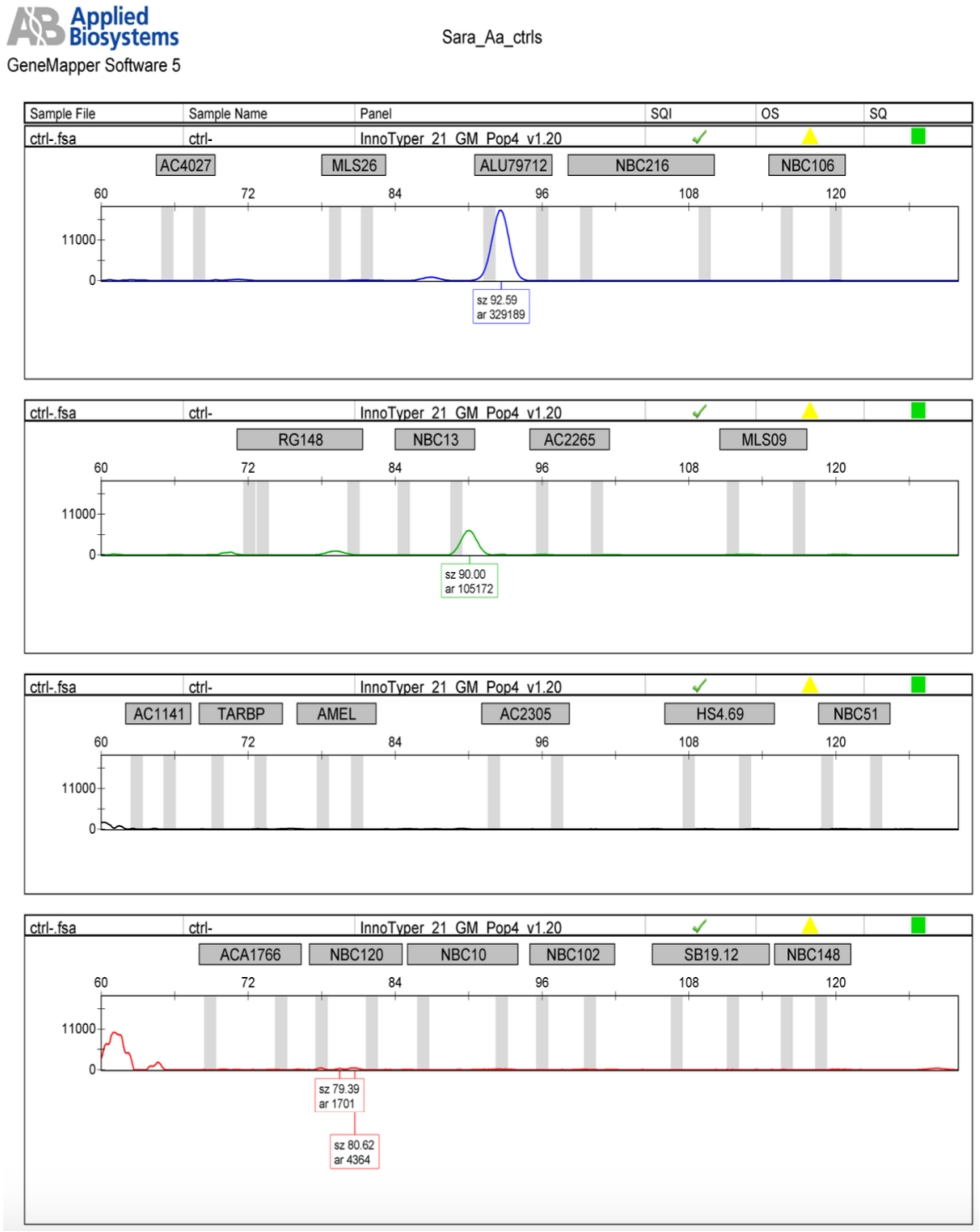


Figure 49 - Negative control from the capillary electrophoresis of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing" (InnoTyper® 21 Human DNA Analysis Kit).

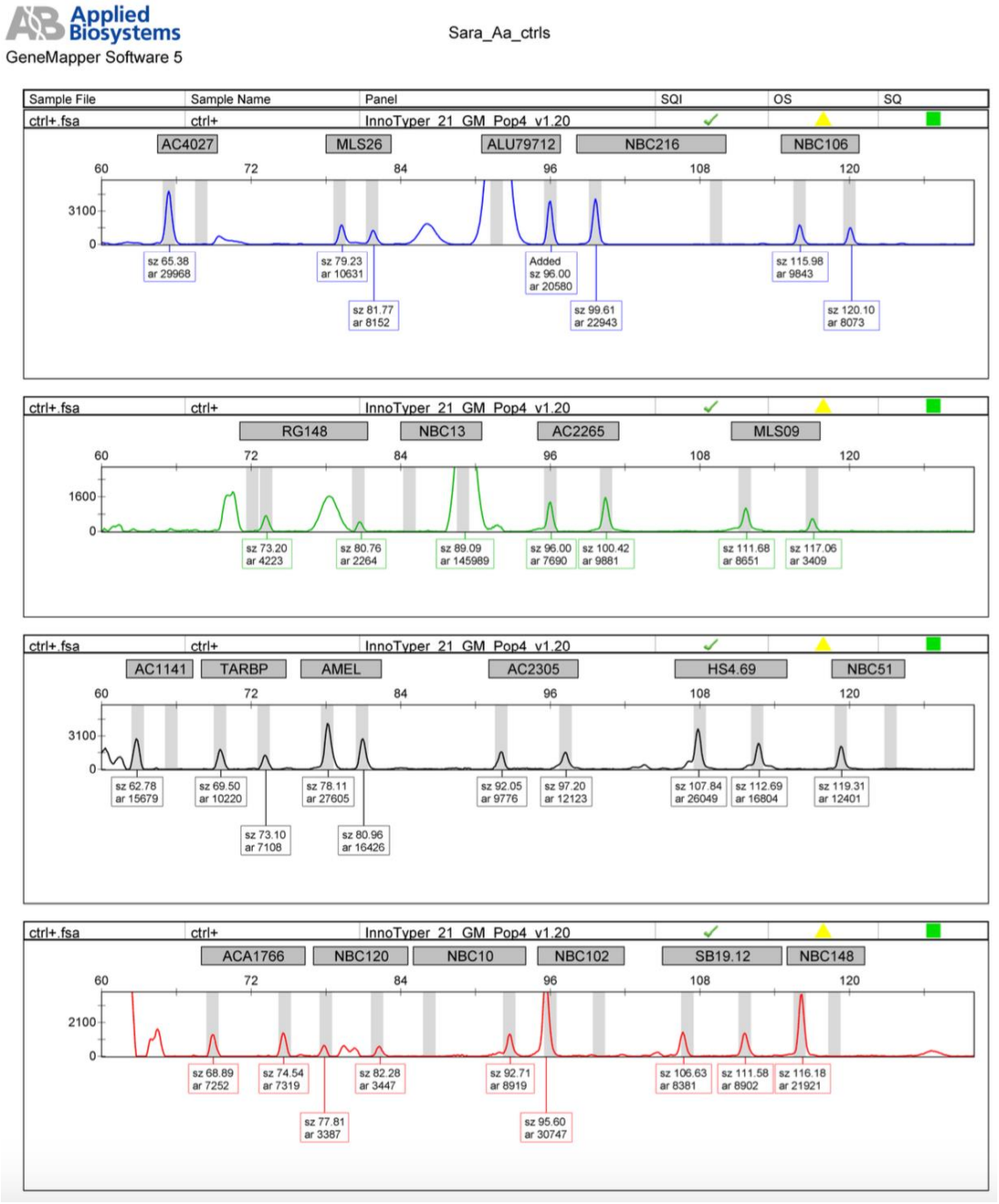


Figure 50 - Genetic profile of the Positive control from the capillary electrophoresis of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing" (InnoTyper® 21 Human DNA Analysis Kit).

Evaluating the number of amplified markers in each genetic profile is was possible to observe that, as expected, all reference samples showed complete genetic profiles (*e.g.* Figure 51).

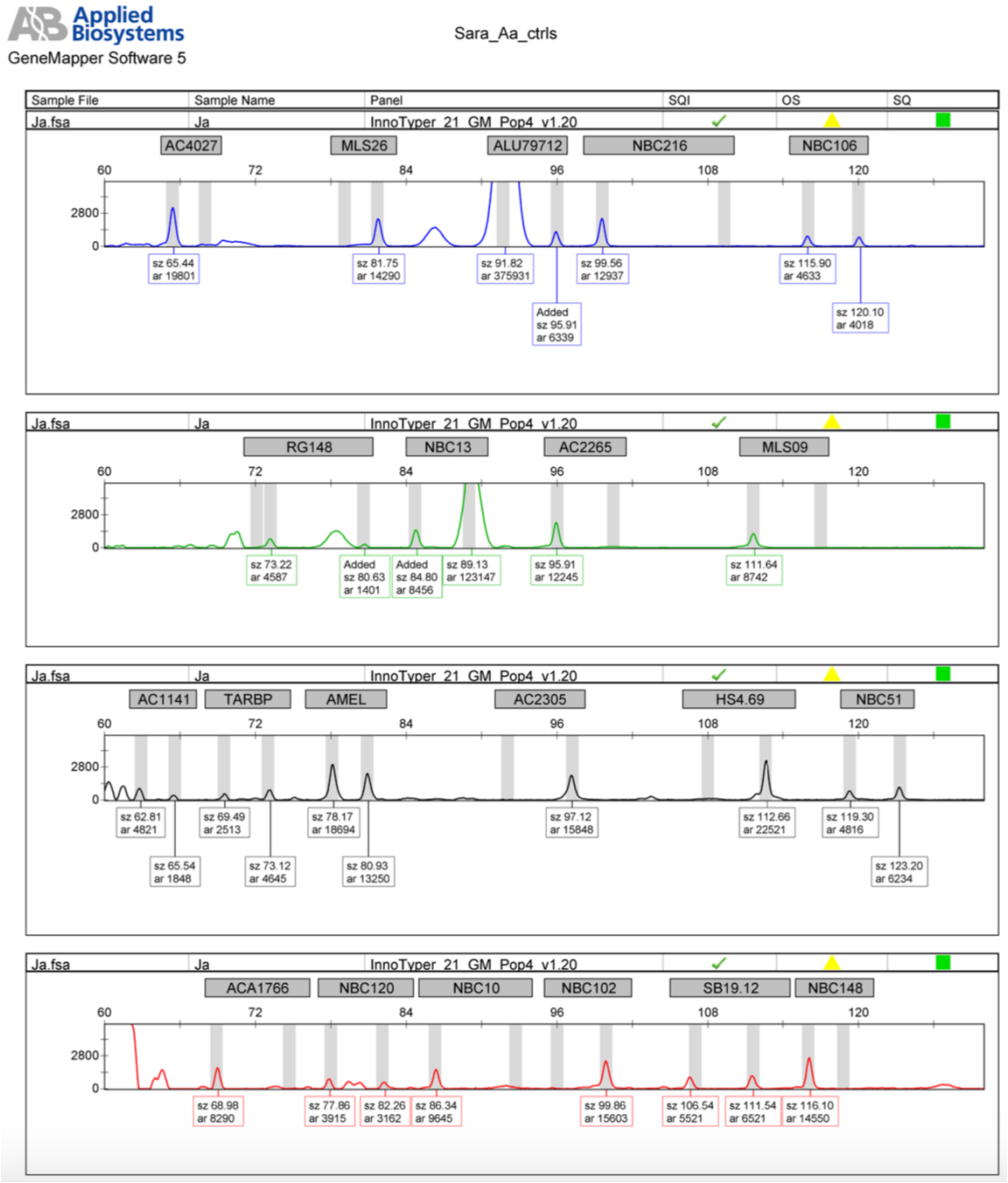


Figure 51 - Genetic profile of the Reference Sample *Ja* from the capillary electrophoresis of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing" (InnoTyper® 21 Human DNA Analysis Kit).

Related to the global questioned samples, despite the ≅ 66.7% expected complete profiles, only ≅ 33.3 % were achieved, being the ≅ 20.8% expected partial profiles all obtained. The expected 12.5% of samples with no profiles were not obtained since most of these profiles turned out to be partial (one complete profile was obtained).

Specifying, into the questioned samples without cyanoacrylate (Figure 52), despite the ≅ 83.3% expected complete profiles, only ≅ 45.8% were achieved (remaining ≅ 37.5% were incomplete), being the 12.5% expected partial profiles all obtained. The expected ≅ 4.2% of samples (one sample) with no profile did not occur since the sample revealed a partial profile.

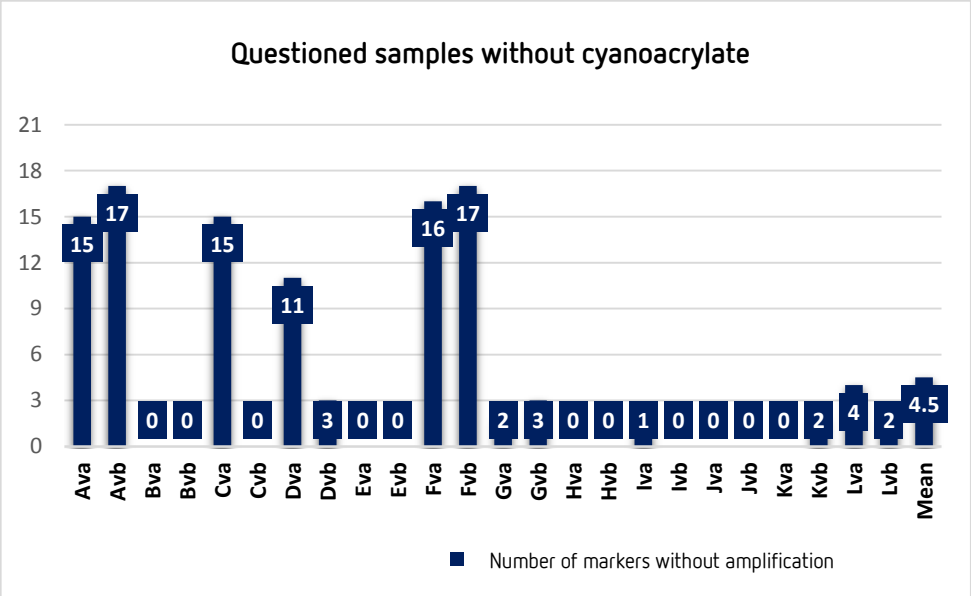


Figure 52 - Number of markers without amplification in the genetic profile of questioned samples without cyanoacrylate from the capillary electrophoresis of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing" (InnoTyper® 21 Human DNA Analysis Kit).

Finally, into the questioned samples developed with cyanoacrylate (Figure 53), despite the 50% expected complete profiles, only ≅ 20.8% were achieved (remaining ≅ 29.2% were incomplete), being the 29.2% expected partial profiles all obtained. The expected ≅ 20.8% of samples with no profiles were not obtained since most of these profiles turned out to be partial (one complete profile was obtained, increasing the percentage of complete profiles to 25%).

Since samples without cyanoacrylate allowed to obtain almost the double of complete profiles when compared with samples developed with cyanoacrylate, it seems that the developement agent, despite not inhibiting the obtaintion of genetic profiles, lead to less recover of profiles in a complete state and, within the partial profiles obtained, lead to amplification of fewer genetic markers (Figure 52, Figure 53). Although using different

methods to obtain genetic profiles, similar findings were already achieved (Bille et al., 2009; Risoluti et al., 2019).

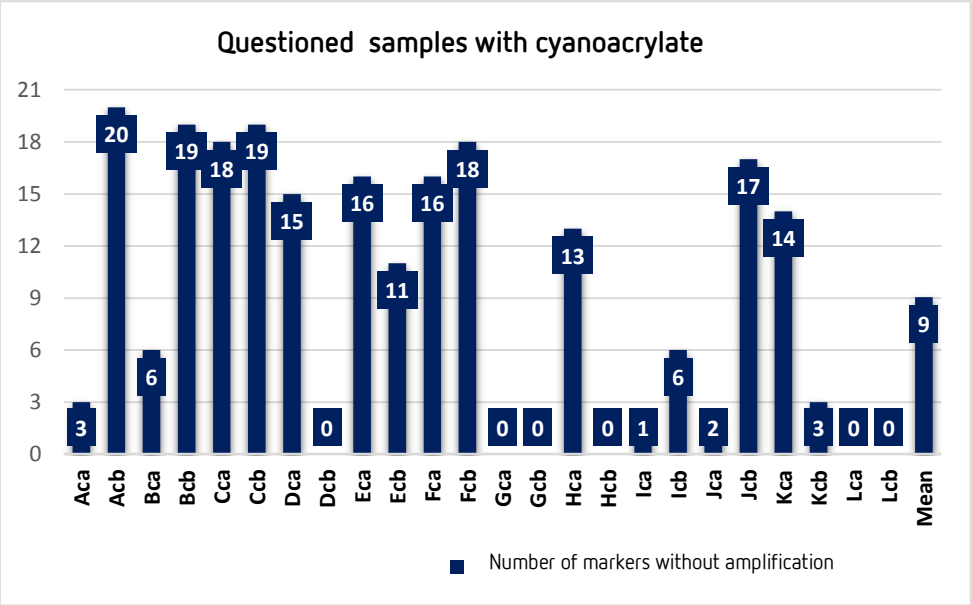


Figure 53 - Number of markers without amplification in the genetic profile of questioned samples developed with cyanoacrylate from the capillary electrophoresis of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing" (InnoTyper® 21 Human DNA Analysis Kit.

It is important to highlight that, when in presence of possible mixtures, it is impossible to truly evaluate the complete state of a genetic profile since Allele/ Locus Drop Outs (ADO/ LDO) can be masked by the presence of alleles from a different donor. Thus it is important to clarify that the concept assumed in this work as "complete profile", for the questioned samples, only means amplification in all markers. Therefore, in the true sense of the concept, the percentages of the complete profiles obtained, could be even fewer in comparison to the expected results.

Since the expected percentages of complete profiles were only thought in relation to the DNA quantity of each sample, it should be the considerable DNA DI that lead to the decrease of the recovered complete profiles. Thus, it seems simple to realize that stipulating the proper amount of DNA to input for amplification could be a difficult achievement when despite having some considerable DNA concentration, the molecule quality in not the desirable. In the present work, only around 50% of the expected complete profiles were

obtained in both groups of questioned samples, what is in accordance with their similar DI. On the other hand, there were also some samples that showed partial profiles while the expectation was to see none. This happening can probably be due to the fact that these samples were out of the Std. concentrations range, therefore their DNA quantity may not have been properly measured, being underestimated.

Correlating fragment analysis with specific data obtained in the qPCR, this quantification method revealed that two samples presented some inhibition, *Lb* and *Hca*. However, only the sample *Hca* revealed a partial profile (DI between 3 and 10) since sample *Lb* revealed a complete profile even having a DI superior to 10 (the great DNA quantity probably overlapped the DI). Additionally, *Cva* and *Ccb* samples did not have their long targets amplified and while the former should had a complete profile, the latter should had a partial one (evaluating by the DNA quantity). In fact, both presented incomplete profiles, being impossible to calculate their DI. There was no sample without revealing genetic profile data.

When wanting to match DNA questioned samples with reference profiles, the detailed electropherograms analysis and interpretation, through individual assessment of each marker genotype, was firstly executed in the reference samples (Table 19), posteriorly in the questioned samples without cyanoacrylate (Table 20), and finally in questioned samples developed with cyanoacrylate (Table 22). The genotype evaluation was performed through comparison with the different alleles of the Allelic Ladder and their specific sizes.

DNA profiles from reference samples (Table 19) presented little background noise. Due to the ALU79712 artefact it was always not possible to assume if the volunteer was homozygous for the N allele or heterozygous having the genotype IN. In this marker, only the genotype II was undoubtly assumed. Related to the NBC13 artefact it was also impossible to perceive if the volunteer was heterozygous having the genotype IN or homozygous for the I allele. In this marker, only the genotype NN was undoubtly assumed.

Additionally, since two samples of the same volunteer were collected, it was possible to observe few ADO (1.8%). Most of the affected samples presented low degradation indexes, although this fact may have been the cause for the observed phenomenon. Low DNA quality may lead specific primers not to bind, not amplifying the respective allele. The phenomenon ADO was observed in both alleles (I or N), being this fact related to their similar sizes. In

Short Tandem Repeat (STR) profiles, that have alleles with significant differences in sizes, the drop out occurs preferably in the longest allele (Hunt, 2009).

Table 19 - Genotypes of the reference samples from the capillary electrophoresis of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing" (I=insertion allele; N=null allele; ADO=allele dropout; *=Insertion allele with higher peak around 92bp, coincident with the marker artefact; +=Null allele with higher peak around 89bp, coincident with the marker artefact; InnoTyper® 21 Human DNA Analysis Kit).

Samples / Markers	Aa	Ab	Ba	Bb	Ca	Cb	Da	Db	Ea	Eb	Fa	Fb	Ga	Gb	Ha	Hb	Ia	Ib	Ja	Jb	Ka	Kb	La	Lb
AC4027	NN	NN	NN	NN	II	II	IN	IN	IN	IN	II	II	IN	IN	IN	IN	II	II	II	II	IN	IN	IN	IN
IIMLS26	IN	IN	IN	IN	NN	NN	NN	NN	II	II	IN	IN	II	II	IN	IN	IN	IN	NN	NN	NN	NN	IN	IN
ALU79712	I*N	I*N	I*N	I*N	I*N	I*N	I*N	I*N	I*N	I*N	I*N	I*N	I*N	I*N	I*N	I*N	I*N	I*N	I*N	I*N	I*N	I*N	II	II
NBC216	II	II	II	II	II	II	IN	IN	II	II	IN	IN	II	II	IN	IN	IN	IN	II	II	IN	IN	IN	IN
NBC106	NN	NN	IN	IN	IN	IN	II	II	NN	NN	IN	IN	IN	IN	II	II	IN	IN	IN	IN	NN	NN	II	II
RG148	NN	NN	II	II	II	II	II	II	IN	I-ADO	II	II	II	II	IN	IN	NN	NN	IN	IN	NN	NN	NN	NN
NBC13	IN+	IN+	NN	NN	IN+	IN+	IN+	IN+	NN	NN	IN+	IN+	IN+	IN+	IN+	IN+	IN+	IN+	IN+	IN+	NN	NN	IN+	IN+
AC2265	II	II	IN	IN	IN	IN	II	II	NN	NN	II	II	II	II	IN	IN	IN	IN	II	II	NN	NN	IN	IN
MLS09	IN	IN	II	II	IN	ADO-N	IN	IN	IN	NN	II	II	II	II	IN	IN	IN	IN	II	II	II	II	IN	IN
AC1141	II	II	II	II	II	II	II	II	II	II	IN	I-ADO	II	II	II	II	IN	IN	II	II	IN	IN	IN	IN
TARBP	NN	NN	IN	IN	NN	NN	ADO-N	IN	IN	IN	IN	IN	IN	IN	NN	NN	NN	NN	IN	IN	IN	IN	II	II
AMEL	XY	XY	XY	XY	XY	XY	XX	XX	XX	XX	XY	XY	XX	XX	XX	XX	XX	XX	XY	XY	XX	XX	XY	XY
AC2305	NN	NN	NN	NN	IN	IN	IN	IN	IN	IN	IN	ADO-N	II	II	II	II	NN	NN	NN	NN	NN	NN	IN	IN
HS4.69	IN	IN	IN	IN	IN	IN	IN	IN	IN	IN	IN	IN	IN	IN	IN	IN	II	II	NN	NN	ADO-N	IN	IN	IN
NBC51	IN	IN	II	II	IN	IN	NN	NN	IN	IN	IN	IN	IN	IN	IN	IN	NN	NN	IN	NN	NN	NN	IN	IN
ACA1766	II	II	II	II	IN	IN	ADO-N	IN	NN	NN	NN	NN	IN	IN	IN	IN	II	II	II	II	II	II	II	II
NBC120	IN	IN	IN	IN	IN	I-ADO	II	II	IN	IN	IN	IN	IN	IN	IN	IN	IN	IN	IN	IN	IN	IN	IN	IN
NBC10	II	II	IN	IN	IN	II	NN	NN	NN	NN	ADO-N	IN	IN	IN	II	II	II	II	II	II	II	II	IN	IN
NBC102	IN	IN	IN	IN	II	II	NN	NN	IN	IN	II	II	II	II	NN	NN	IN	IN	II	II	NN	NN	IN	IN
SB19.12	NN	NN	NN	NN	IN	IN	II	II	NN	NN	NN	NN	IN	IN	IN	IN	NN	NN	IN	IN	NN	NN	NN	NN
NBC148	IN	IN	II	II	II	II	II	II	IN	IN	II	II	II	II	II	II	II	II	II	II	II	II	II	II

Specifically observing the marker *Amel* of the sample *Kb*, which presented HEX amplification in the qPCR, due to a possible saliva exchange, it was now confirmed that the volunteer was a female (3200 RFUs), not being exhibited the Y chromosome. This fact corroborates the

higher sensitivity of the InnoQuant® HY-R to detect the Y chromosome (two multi-copy targets) when compared with the InnoTyper® 21 (one target).

Table 20 – Genotypes of the questioned samples without cyanoacrylate from the capillary electrophoresis of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing" (I=insertion allele; N=null allele; ADO=allele dropout; -=locus drop out; *=Insertion allele with higher peak around 92bp, coincident with the marker artefact; +=Null allele with higher peak around 89bp, coincident with the marker artefact; ►=peak interpretation doubt; InnoTyper® 21 Human DNA Analysis Kit).

Samples / Markers	Ava	Avb	Bva	Bvb	Cva	Cvb	Dva	Dvb	Eva	Evb	Fva	Fvb	Gva	Gvb	Hva	Hvb	Iva	Ivb	Jva	Jvb	Kva	Kvb	Lva	Lvb
AC4027	-	-	II	II	-	IN	-	II	II	II	-	-	IN	IN	IN	IN	II	II	IN	IN	IN	IN	II	IN
IIMLS26	-	-	IN	IN	-	II	-	II	NN	NN	-	-	IN	IN	IN	IN	NN	NN	II	II	NN	NN	NN	NN
ALU79712	II	II	I*N	I*N	II	I*N	II	I*N	I*N	I*N	-	II	I*N*	I*N	I*N	I*N	I*N	I*N	I*N	I*N	I*N	I*N	I*N	I*N
NBC216	-	-	IN	IN	-	II	-	II	II	IN	-	-	IN	IN	II	II	II	II	IN	II	IN	IN	II	IN
NBC106	-	-	IN	IN	-	IN	-	NN	IN	IN	-	-	II	II	NN	NN	IN	IN	NN	NN	NN	NN	II	II
RG148	-	-	IN	IN	-	IN	-	-	II	II	-	-	-	-	NN	NN	II	II	IN	IN	NN	-	-	-
NBC13	II	-	IN*	IN*	-	IN*	NN	NN	IN*	IN*	-	-	NN	NN	IN*	IN*	IN*	IN*	IN*	IN*	NN	NN	NN	NN
AC2265	NN	NN	II	II	-	II	NN	IN	II	II	-	NN	II	IN	II	II	IN	IN	IN	IN	NN	NN	II	IN
MLS09	II	II	II	II	II	II	II	II	II	II	II	II	II	II	IN	IN	IN	IN	IN	IN	II	II	I►N	II
AC1141	-	-	IN	IN	-	II	II	II	II	IN	-	-	II	-	II	II►	II	II	II	II	II	II	II	II
TARBP	-	-	IN	IN	II	IN	-	NN	II	IN	II	-	IN	II	NN	NN	NN	NN	IN	IN	IN	IN	-	II
AMEL	-	-	XY	XY	-	XX►	XY►	XX	XY	XY	-	-	XY►	XY	XY	XY	XY	XY	XX	XY	XX	XX	XX	XX
AC2305	IN	-	IN	IN	-	II	-	-	NN	NN	-	-	NN	NN	NN	NN	NN	IN	IN	IN	NN	NN	-	IN
HS4.69	IN	IN	IN	IN	IN	IN	IN	IN	IN	IN	NN	IN	IN	IN	IN	IN	IN	IN	IN	IN	NN	IN	IN	IN►
NBC51	-	-	IN	IN	IN	IN	II	II	IN	IN	-	-	NN	IN	IN	IN	I►N	IN	IN	IN	NN	NN	NN	NN
ACA1766	-	-	NN	NN	-	IN	II	IN	IN	IN	-	-	-	IN	II	II	II	IN	NN	NN	II	-	NN	II
NBC120	-	-	IN	IN	IN	IN	-	II	IN	IN	-	-	II	IN	IN	I►N	II	II	IN	IN	I►N	II	II	II
NBC10	-	-	NN	NN	-	IN	-	-	II	II	II	-	NN	-	II	II	-	IN	NN	NN	II	II	-	-
NBC102	-	-	IN	II	-	IN	NN	IN	IN	IN	-	-	IN	NN	IN	IN	IN	IN	IN	IN	NN	NN	NN	NN
SB19.12	-	-	NN	NN	-	IN	-	NN	IN	IN	NN	-	IN	NN	NN	NN	IN	IN	NN	NN	NN	NN	II►	II►
NBC148	-	-	II	II	-	II	-	II	II	II	-	-	II	II	IN	IN	II	II	IN	IN	II	II	II►	II

Most of the DNA profiles from questioned samples without cyanoacrylate (Table 20) presented the already referred issue in the ALU79712 and NBC13 markers. Additionally, and as it was expected due to the existence of partial profiles and due to the higher DI, instead of presenting ADO, some markers presented considerable LDO (20.83%; Figure 52), meaning the inexistence of amplification on both alleles. The electropherogram peaks also presented lower height and lower configuration quality when compared to reference

samples. Therefore, some flagged doubts appeared when evaluating the existence or non-existence of alleles.

The samples *Ava, Avb*, *Dva*, *Dvb, Fva, Fvb, Gva, Gvb, Lva and Lvb* presented LDO in a significant number of markers, some of them including in the *AMEL* locus. Therefore, it was not possible to perform sex determination in those cases (*Ava*, *Avb*, *Fva* and *Fvb*), neither to establish a match with reference samples in all of them.

Despite all the emerged difficulties, when evaluating and comparing profiles, it was possible to find matches between some questioned and reference samples (Table 21), that were posteriorly confirmed as correct by the PI.

Table 21 - Correspondence between questioned samples without cyanoacrylate and respective reference samples of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing" (LDO=locus drop out).

Questioned samples without cyanoacrylate	Reference sample	Genotype divergence
Bva	Fa and Fb	Bvb: ACA1766
Bvb		
Cva	Ga and Gb	Cva: ALU79712 + TARBP
Cvb		Cvb: RG148 + NBC102
Eva	Ja and Jb	Eva: TARBP
Evb		Evb: NBC216
		Both: RG148 + HS4.69 + ACA1766
Hva	Aa and Ab	None
Hvb		
Iva	Ca and Cb	Iva: AC2305
Ivb		Both: HS4.69 + NBC102
Jva	Ea and Eb	Jva: NBC216
Jvb		Jvb: AMEL
		Both: AC2265
Kva	Ka and Kb	Kva: HS4.69
Kvb		Kvb: NBC120 + two LDO (RG148 and ACA1766)
		Both: AC1141

Trough genotype comparison it was possible to find matches in fourteen samples (58.3%), in a universe of twenty four samples (Figure 54).

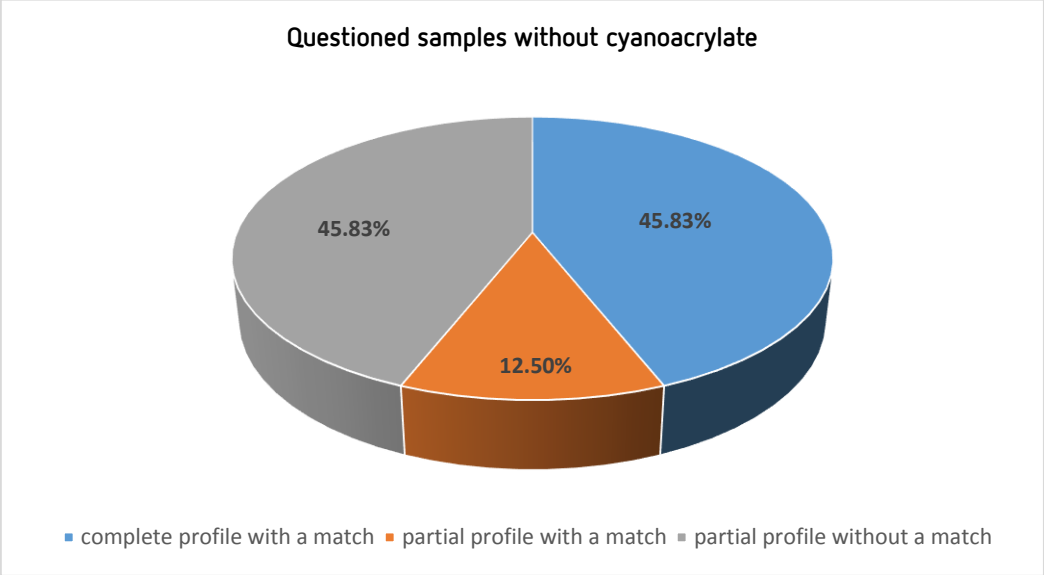


Figure 54 - Matching rates of the profiles (complete and partial) from the questioned samples without cyanoacrylate of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing".

It is important to reinforce that samples *Ava*, *Cva*, *Cvb*, *Jva*, *Kva*, *Kvb*, *Lva* and *Lvb* which presented HEX amplification in the qPCR, being female volunteers, did not exhibit Y chromosome in the fragment analysis. The same reason is again speculated, when non related with drop out phenomenon: the presence of contamination in enough quantity to be detected by the InnoQuant® HY-R kit but not enough to be detected by InnoTyper® 21 kit. In sample *Jvb*, a female, contamination was clearly assumed since HEX was amplified in the qPCR, and also exhibited Y chromosome in the fragment analysis. In this case, the match was assumed due to the high coincidence of the other markers and also, influenced by the previous knowledge that it came from the same volunteer as the sample *Jva.* The volunteer *I*, that drove the steering wheel previously to the volunteer *J* was a male, so probably this was a result of inefficient cleaning between driving.

Finally, related to samples without cyanoacrylate, although not being so discrepant again, it is important to draw attention to the fact that, as it happened with the DNA quantity that was higher on the left side of the steering wheel, most of the obtained complete genetic profiles were from the same side (Figure 55). On the other hand, while the DNA quantity was higher in females, the obtention of complete profiles was higher in males (Figure 56).

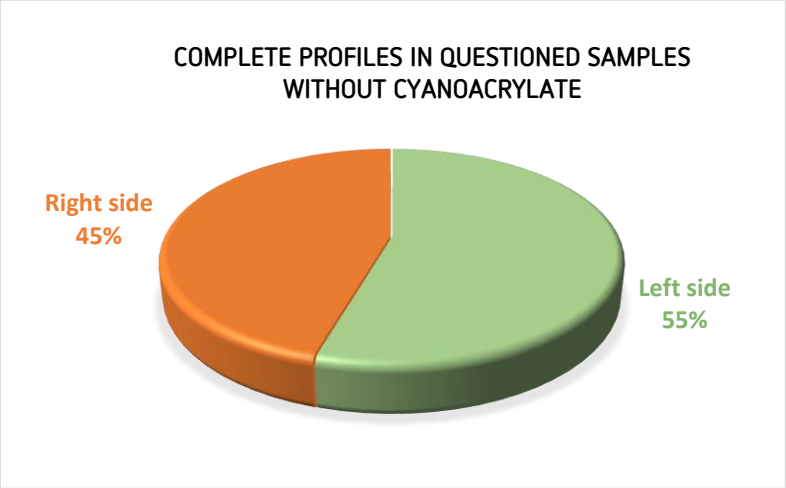


Figure 55 – Frequency of complete profiles, in samples without cyanoacrylate, according to the side of steering wheel.

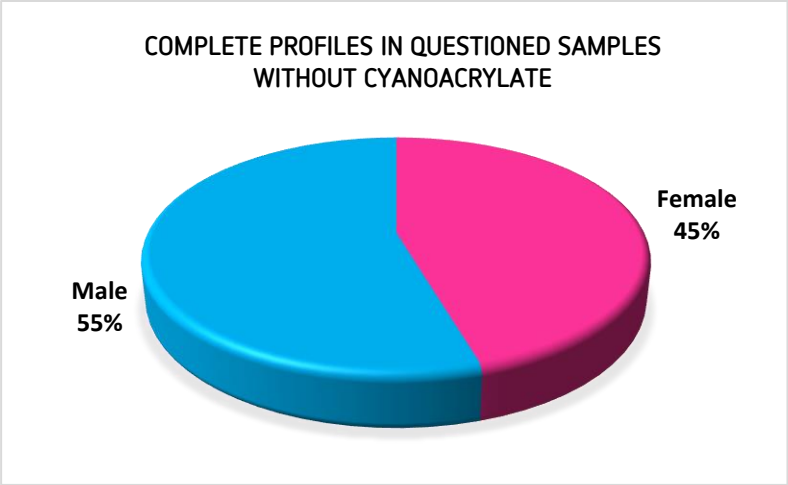


Figure 56 – Percentage of complete profiles, in samples without cyanoacrylate, in both sexes.

Related to DNA profiles of the questioned samples developed with cyanoacrylate (Table 22), most of them also presented the previous referred issue in the ALU79712 and NBC13 markers. Additionally, and as it was also expected due to the highest existence of partial profiles and due to the significant DI, an increased number of markers presented LDO (43.05%, Figure 53). The electropherogram peaks also presented lower height and lower configuration quality when compared to reference samples. Therefore, a greater number of doubts arose when evaluating the alleles genotypes.

The samples *Aca*, *Acb*, *Bca*, *Bcb*, *Cca*, *Dca*, *Ccb*, *Eca*, *Ecb*, *Fca*, *Fcb*, *Ica*, *Icb*, *Jca*, *Jcb*, *Kca* and *Kcb* presented LDO in a significant number of markers, some of them including LDO in *AMEL* locus. Therefore, it was not possible to perform sex determination in those cases (*Acb*, *Bcb*, *Cca*, *Ccb*, *Dca*, *Fca*, *Fcb*, *Jcb* and *Kca*), neither to establish a match with reference samples in all of them. Even previously knowing that sample *Dca* came from the same volunteer than sample *Dcb*, this match was not considered since it was not possible to establish the match only evaluating the genetic profiles.

As it happened in the samples without cyanoacrylate, despite all the emerged and more significant difficulties, when evaluating and comparing profiles, it was steel possible to find few matches between questioned and reference samples (Table 23) that were posteriorly confirmed as correct by the PI.

Table 22 - Genotypes of the questioned samples developed with cyanoacrylate from the capillary electrophoresis of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing" (*=Insertion allele with higher peak around 92bp, coincident with the marker artefact; +=Null allele with higher peak around 89bp, coincident with the marker artefact; InnoTyper® 21 Human DNA Analysis Kit).

Samples / Markers	Aca	Acb	Bca	Bcb	Cca	Ccb	Dca	Dcb	Eca	Ecb	Fca	Fcb	Gca	Gcb	Hca	Hcb	Ica	Icb	Jca	Jcb	Kca	Kcb	Lca	Lcb
AC4027	IN	-	NN	-	-	-	-	IN	-	II	-	-	II	II	-	II	IN	IN	IN	-	-	II	IN	IN
IIMLS26	NN	NN	NN	NN	NN	NN	-	NN	-	-	-	-	IN	IN	-	IN	IN	NN	IN	-	-	NN	NN	NN
ALU79712	I*N	-	I*N	-	-	-	II	I*N	II	II	II	II	I*N	I*N	II	I*N	I*N	I*N	I*N	-	II	I*N	I*N	I*N
NBC216	II	-	II	-	-	-	-	IN	-	-	-	-	IN	IN	-	IN	II	II	IN	-	-	IN	IN	IN
NBC106	-	-	-	-	-	-	-	II	-	-	-	-	IN	IN	-	IN	IN	-	IN	-	-	II	NN	NN
RG148	-	-	-	-	-	-	-	II	-	-	-	-	IN	II	-	NN	II	-	II	-	-	-	NN	NN
NBC13	Ii	-	-	-	-	-	-	IN	-	NN	NN	-	IN+	IN+	NN	IN+	IN+	NN	NN	-	-	-	NN	NN
AC2265	II	-	-	-	-	-	-	II	NN	NN	-	-	II	II	NN	IN	II	II	IN	NN	NN►	IN►	NN	NN
MLS09	-	-	-	-	-	-	II	II►	II►	II►	II	II	II►	II►	II	IN	IN►	IN	IN	II	II	II	II	II
AC1141	IN	-	II	-	-	-	II	II	-	II	-	-	IN	II	II	IN	II	II	II	-	-	II	IN	IN
TARBP	IN	-	NN	II	-	NN	II	IN	-	-	II	-	IN	IN	-	NN	IN	-	IN	-	II	IN	IN	IN
AMEL	XX	-	XX	-	-	-	-	XX	-	XX	-	-	XY	XY	XX	XX	XY	XY	XX	-	-	XX	XX	XX
AC2305	IN	-	-	-	-	-	-	II	-	-	-	-	IN	IN	-	NN	NN	NN	-	-	-	-	NN	NN
HS4.69	IN	-	II►	-	-	-	IN	IN►	-	IN	NN►	NN	IN	IN	II	II	IN	IN	IN	IN	IN	IN	IN	IN
NBC51	II	-	II	-	NN	-	NN►	NN	IN	-	-	-	IN	IN	-	NN	IN	-	IN	-	NN	IN	NN	NN
ACA1766	IN	-	II	-	-	-	-	NN	-	-	-	-	NN	NN	-	II	NN	-	IN	-	-	NN	II	II
NBC120	II	-	IN	-	-	-	-	IN	NN	NN	-	-	IN	IN	NN	NN	IN	IN	II	-	-	NN	IN	IN
NBC10	NN	-	NN	-	-	-	-	NN	-	-	-	-	NN	NN►	-	II	-	-	NN	NN	II	II	II	II
NBC102	IN	-	IN	-	-	-	-	NN	-	NN	-	-	IN	II	-	IN	IN	NN	IN	-	-	IN	NN	NN
SB18.12	II	-	NN	-	-	-	-	II	-	-	-	-	NN	NN	-	NN	IN	NN	NN	-	-	IN	NN	NN
NBC148	II	-	II	-	NN	N	-	II	-	-	-	-	II	II	-	IN	IN	II	-	-	-	II	II	II

Table 23 - Correspondence between questioned samples developed with cyanoacrylate and respective reference samples of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing" (LDO=locus drop out).

Questioned samples developed with cyanoacrylate	Reference sample	Genotype divergence
Dcb	Da and Db	Dcb: MLS09 + AC2305 + ACA1766 + NBC120
Gca	Fa and Fb	Gca: RG148
Gcb		Gcb: AC1141
Hca	Ia and Ib	Hca: ALU79712 + NBC13 + AC2265 + MLS09 + AC1141 + LDO in NBC148
Hcb		Hcb: NBC148 Both: NBC120
Lca	Ka and Kb	None
Lcb		

Trough genotype comparison it was possible to find matches in seven samples (≅ 29.17%), in a universe of twenty four samples (Figure 57).

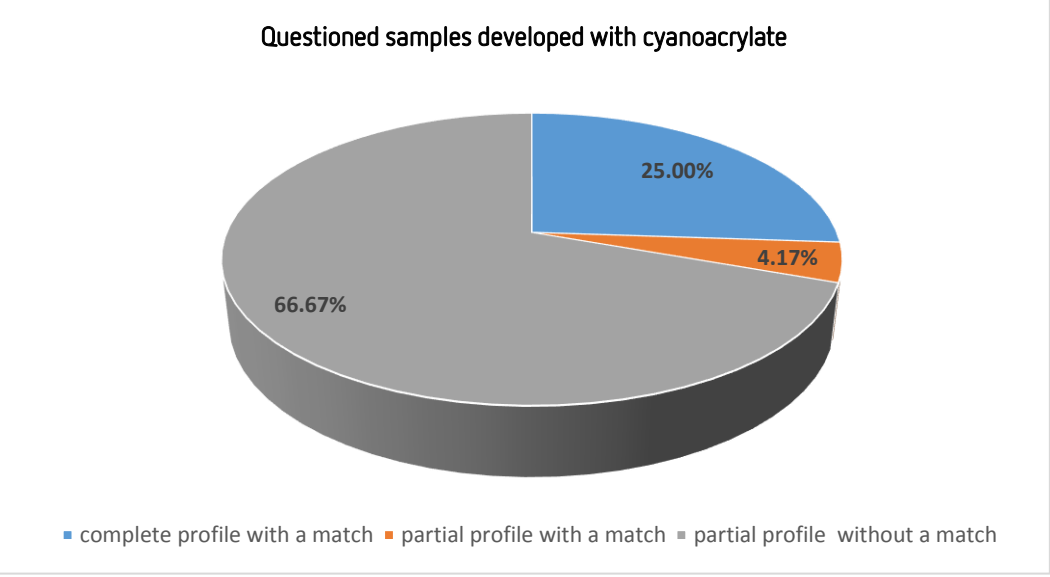


Figure 57 - Matching rates of the profiles (complete and partial) from the questioned samples developed with cyanoacrylate of the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing".

It is again important to reinforce that samples *Bca*, *Bcb*, *Dca*, *Eca*, *Hca*, *Hcb*, *Jca*, *Jcb*, *Lca* and Lcb which presented HEX amplification in the qPCR, being female volunteers, did not exhibit Y chromosome in the fragment analysis. The possible reasons for this happening were already previously explained. On the other hand, samples *Aca*, *Acb* and *Cca*, being male volunteers, did not present HEX amplification in the qPCR and even in the fragment analysis the Y chromosome was not exhibited. This can probably be related to few target quantities (even not enough for InnoQuant® HY-R sensitivity) and/ or possible drop out phenomenon in the second case. Male Samples Aca and *Kcb* were genotypically classified as homozygous (XX) for the marker *Amel* (both alleles around 500 RFU), which also hampered the achievement of the aimed matches.

Finally, related to samples developed with cyanoacrylate, although not being so discrepant again, it is important to draw attention to the fact that as it happened to the DNA quantity that was higher on the left side of the steering wheel, most of the complete genetic profiles were again from the same side (Figure 58). On the other hand, while the DNA quantity was higher in the female sex, the obtention of complete profiles was again higher in the male sex (Figure 59).

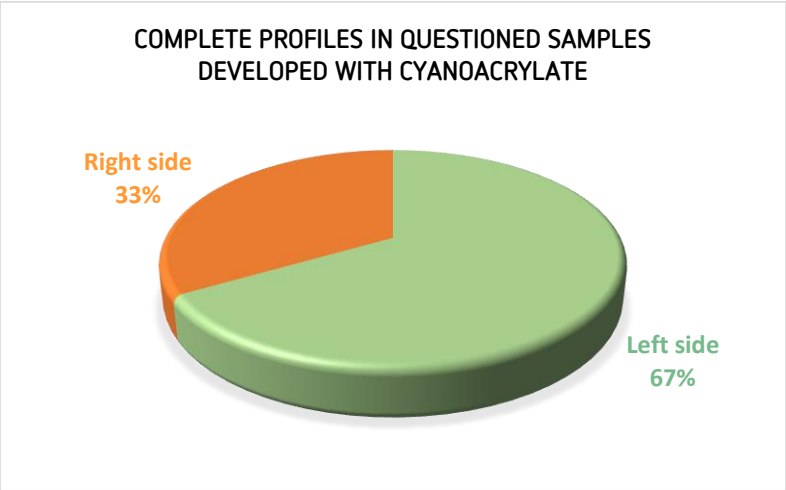


Figure 58 - Percentage of complete profiles, in samples developed with cyanoacrylate, according to the side of steering wheel.

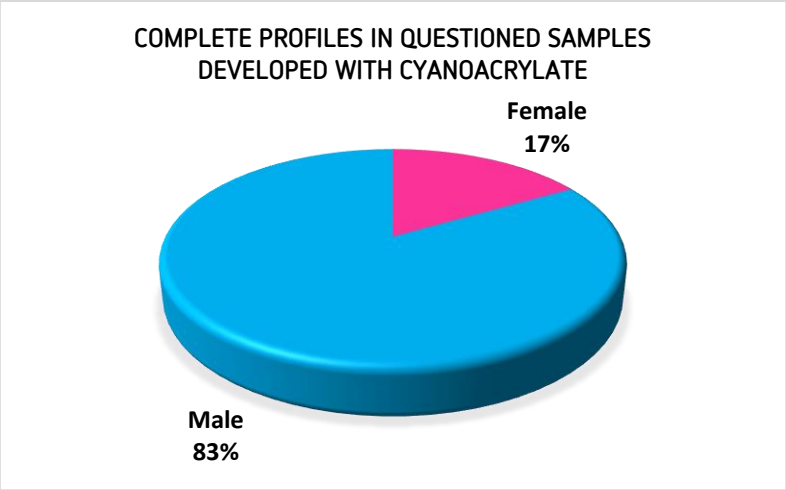


Figure 59 – Percentage of complete profiles, in samples without cyanoacrylate, in both sexes.

Ending the interpretation and discussion of all genetic profiles, it is important to point out that it may be assumed that the non-correspondence between some genotypes of the same marker, in genetic profiles of the same car driver, when not related with the drop out phenomenon, can probably be due to some contamination not being the cleaning steering wheels method 100% effective. Genetic profiles could be revised and analysed in each case of marker discordance evaluating peak height ratios and interpreting the possible existence of mixtures. However, peak height ratios vary also when in presence of low DNA quantity (Brown et al., 2017) and, more important, that was not the purpose of the current work. In the current work it was aimed to identify who drove the steering wheels and even in the presence of possible mixtures (with the additional possibility of having more than one contributor), it was possible to blindly achieve a considerable number of profile correspondences. The presented mock scene ended up mimicking a closer real-life scenario since in real life there will be no cases where only one person has driven a specific car. Therefore, it was shown that in real life cases where mixtures are present, having also the dominance of the perpetrator DNA, the establishment of genetic matches between reference profiles and questioned samples can be a reality, even using InnoTyper® 21, a bi-allelic system, for typing DNA.

Lastly, despite being influenced by the state of the questioned samples, once the InnoTyper® 21 is a relatively new marketed kit, it seemed important to evaluate the percentage of amplification efficiency of each marker, being without or under the influence

of cyanoacrylate (Table 24). It was observed that, independently from the initial state of the samples, the most and the least efficient markers were the same. The two most efficient markers were MLS09 and HS4.69, being the marker RG148 the least efficient. Therefore, this observation probably discloses the primers which have the best and the worst design parameters, having a great impact on the amplification efficiency of each marker.

Table 24 - Percentage of amplification efficiency of each marker of the InnoTyper® 21 kit when used in the research project "Human Traces from Car Inner Surfaces – Broadening the Application of Genetic Policing" (*=marker with artefact peak).

Markers / Samples type	AG4077	MLS16	AU79712*	N8C216	N8C106	RG148	N8C17*	AC2265	MLS09	AC1141	TARBP	AMEL	AC2305	HS4.69	N8C51	AG47N6	N8C120	N8C10	N8C132	SGB12	N8C146
Efficiency without cyanoacrylate (%)	75	75		75	75	54.2		91.7	100	75	79.2	79.2	66.7	100	87.5	70.8	79.2	62.5	79.2	79.2	75
Efficiency with cyanoacrylate (%)	54.2	66.7		50	37.5	33.3		66.7	75	62.5	66.7	62.5	37.5	79.2	62.5	50	62.5	50	54.2	54.2	50

V. CONCLUSIONS

Acting in a mock scene, it was aimed to assess the difficulties of achieving the identification of car drivers, based on the biological traces that can be left on the steering wheels, namely lophoscopic and DNA traces, through the use of cyanoacrylate fuming and recent genetic retrotransposon-based kits, contributing to the establishment of profitable protocols of action by the police forces.

Lophoscopic expertise revealed that using cyanoacrylate as a development agent, it was possible to identify a third of the car drivers, this is, four volunteers (1, 2, 10 and 12). Additionally, despite the limited number of volunteers involved in the current work, and despite not being one of the main aims of the present study, it was also possible to perceive that there is a need for population studies evaluating the predominance of the dactyloscopic types and minutiae, in order to be able to distinguish specific populations, narrowing the possibilities of identification when there are no suspicions of who a person might be.

Genetic expertise revealed that combining the phenol-chloroform method to extract and purify DNA with the InnoQuant HY-R® Kit to posteriorly quantify and the InnoTyper® 21 Kit to perform fragment analysis, it was possible to identify two thirds of the car drivers, this is, eight volunteers (1, 3, 4, 6, 9, 10, 12). However, when genetic expertise took place only after using cyanoacrylate, trying to observe lophoscopic marks first, the number of car drivers identified decreased to four (5, 10, 11, 12). Thus, in the current research it was observed that the use of cyanoacrylate did not seem to inhibit both kit reagents contributing only to the reduced amount of extracted DNA during the phenol-chloroform extraction, resulting in worse quality (fewer amplified markers) of the obtained genetic profiles, hampering the establishment of genetic matches. The execution of both types of expertise seems to indicate that the amount of sweat and sebum that is released (contributing to form lophoscopic marks), should not be, at least always, directly correlated to the amount of the flacking cells (contributing for DNA analysis), since only half of the car drivers identified through lophoscopic expertise was also identified through genetic expertise.

Additionally, through genetic expertise it was possible to observe that higher DNA quantities were obtained from the left side of the steering wheels (with and without the use of

cyanoacrylate), resulting also in higher quality of the obtained genetic profiles, which lead to the achievement of more genetic matches.

Knowing that, as an internal procedure, forensic experts from the Polícia Judiciária normally collect genetic traces from the steering wheels first and only afterwards perform lophoscopic analysis, the current work seems to scientifically validate and reinforce the existent protocol of action. Considering our study, when performing lophoscopic analysis first and then genetics, it was possible to identity fewer car drivers, having also the disadvantage related to time consuming for the resolution of the fake forensic cases and the need of more human resources.

With the obtained results, it is believed that, nowadays, when police forces met with this type of needs, efforts should be focused in the execution of genetic analysis. Additionally, it is now also believed that if more attention is paid to the left side of the steering wheel, a higher probability of achieving genetic matches should be obtained.

This work leaves the curiosity of knowing the success rate of the genetic kits that are nowadays currently used by the Polícia Judiciária, if applied in this type of challenging surfaces. Thus, in the future, comparative studies should be perform using both kits within the same context to ascertain which are the best DNA targets to be considered in forensic critical samples, as the case of Touch DNA. Additionally, and due to the expensive cost of genetics, research should also be made in order to find a suitable development agent to be applied in this type of surfaces, possibly leading to the discovery of new development agents. Directing forensic investigation in these two paths, will contribute to the development of both areas of expertise, increasing the success on forensic cases resolution, having simultaneously an inhibiting effect whenever anyone thinks of committing an illicit act.

VI. REFERENCES

Aditya S, Kalpana D, Bhattacherjee S and Sharma AK (2011) Low copy number DNA typing of touched evidence by miniSTRs- A case study. *Indian Journal of Biotechnology* **10**:147-149.

Alessandrini F, Cecati M, Pesaresi M, Turchi C, Carle F and Tagliabracci A (2003) Fingerprints as evidence for a genetic profile: morphological study on fingerprints and analysis of exogenous and individual factors affecting DNA typing. *Journal Forensic Science* **48**:586-592.

Alonso A, Andelinovic S, Martin P, Sutlovic D, Erceg I, Huffine E, de Simon LF, Albarran C, Definis-Gojanovic M, Fernandez-Rodriguez A, Garcia P, Drmic I, Rezic B, Kuret S, Sancho M and Primorac D (2001) DNA typing from skeletal remains: evaluation of multiplex and megaplex STR systems on DNA isolated from bone and teeth samples. *Croatian Medical Journal* **42**:260-266.

Auton A, Abecasis GR, Altshuler DM, Durbin RM, Abecasis GR, Bentley DR, Chakravarti A, Clark AG, Donnelly P, Eichler EE, Flicek P, Gabriel SB, Gibbs RA, Green ED, Hurles ME, Knoppers BM, Korbel JO, Lander ES, Lee C, Lehrach H, Mardis ER, Marth GT and McVean GA (2015) A global reference for human genetic variation. *Nature* **526**:68.

Ayarpadikannan S and Kim H-S (2014) The impact of transposable elements in genome evolution and genetic instability and their implications in various diseases. *Genomics & Informatics* **12**:98-104.

Baroni A, Buommino E, De Gregorio V, Ruocco E, Ruocco V and Wolf R (2012) Structure and function of the epidermis related to barrier properties. *Clinics in Dermatology* **30**:257-262.

Bille T, Cromartie C and Farr M (2009) Effects of Cyanoacrylate Fuming, Time After Recovery, and Location of Biological Material on the Recovery and Analysis of DNA from Post-Blast Pipe Bomb Fragments. *Journal of Forensic Science* **54**:1059-1067.

Brown H, Thompson R, Murphy G, Peters D, La Rue B, King J, Montgomery AH, Carroll M, Baus J, Sinha S, Wendt FR, Song B, Chakraborty R, Budowle B and Sinha SK (2017) Development and validation of a novel multiplexed DNA analysis system, InnoTyper 21. *Forensic Science International: Genetics* **29**:80-99.

Butler JM (2010) Chapter 6 - DNA Quantitation, in *Fundamentals of Forensic DNA Typing* (Butler JM ed) pp 111-124, Academic Press, San Diego.

Butler JM (2012) Chapter 6 - Capillary Electrophoresis: Principles and Instrumentation, in *Advanced Topics in Forensic DNA Typing: Methodology* (Butler JM ed) pp 141-165, Academic Press, San Diego.

Cainé L (2010) Entomologia Forense: Identificação Genética de Espécies em Portugal. , Faculdade de Medicina da Universidade de Coimbra, Coimbra, Portugal.

Carvalho MR (2010) Single Nucleotide Polymorphisms (SNPs) com aplicação forense, in *Genética Forense : Perspectivas da identificação genética* (Pessoa UF ed) pp 187-197, Universidade Fernado Pessoa, Porto.

Cordaux R and Batzer MA (2009) The impact of retrotransposons on human genome evolution. *Nature Reviews Genetics* **10**:691-703.

Correia PNO (2008) Lofoscopia - Uma breve introdução, in *CSI Criminal* (Pessoa UF ed) pp 143-156, Universidade Fernando Pessoa, Porto, Portugal.

Ebling F and Montagna W (2016a) Human skin, Retrieved 18/5/2019, Encyclopædia Britannica, inc., England.

Ebling F and Montagna W (2016b) The Skin Surface, Retrieved 18/05/2019, Encyclopædia Britannica, inc., England.

Fawcett AS (1970) The Role of the Footmark Examiner. *Journal of the Forensic Science Society* **10**:227-244.

Finnegan DJ (2012) Retrotransposons. *Current Biology* **22**:R432-R437.

Funabashi K, Monteiro AC, Moraes D, Rocha M, Moreira PC and Iwamura E (2009) A importância da identificação humana nos desastres de massa naturais, acidentais ou provocados: uma abordagem multidisciplinar. *Saúde, Ética & Justiça* **14 (2)**:54-64.

Geada HS (2010) DNA mitocondrial. Aplicação ao estudo do DNA humano, in *Genética Forense: Perspectivas da identificação genética* (Fessoa UF ed) pp 139-161, Univeridade Fernando Fessoa, Porto, Portugal.

Girod A, Ramotowski R, Lambrechts S, Misrielal P, Aalders M and Weyermann C (2016) Fingermark age determinations: Legal considerations, review of the literature and practical propositions. *Forensic Science International* **262**:212-226.

Goecker ZC, Swiontek SE, Lakhtakia A and Roy R (2016) Comparison of Quantifiler Trio and InnoQuant human DNA quantification kits for detection of DNA degradation in developed and aged fingerprints. *Forensic Science International* **263**:132-138.

Gouveia N, Brito P, Bogas V, Serra A, Bento AM, Lopes V, Balsa F, Sampaio L, São Bento M, Cunha P and Porto MJ (2017) The effect of different levels of degradation and DNA concentrations on the quality of genetic profiles. *Forensic Science International: Genetics Supplement Series* **6**:428-429.

Griffiths A, R.Wessler S, B.Carroll S and Doebley J (2015) *Introduction to genetic analysis*, W.H. Freeman & Company, New York.

Gutiérrez-Redomero E, Alonso-Rodríguez C, Hernández-Hurtado LE and Rodríguez-Villalba JL (2011) Distribution of the minutiae in the fingerprints of a sample of the Spanish population. *Forensic Science International* **208**:79-90.

Hancks DC and Kazazian HH, Jr. (2010) SVA retrotransposons: Evolution and genetic instability. *Seminars in Cancer Biology* **20**:234-245.

Hormozdiari F, Alkan C, Ventura M, Hajirasouliha I, Malig M, Hach F, Yorukoglu D, Dao P, Bakhshi M, Sahinalp SC and Eichler EE (2011) Alu repeat discovery and characterization within human genomes. *Genome Research* **21**:840-849.

Horswell J (2004) *The Practice Of Crime Scene Investigation*, CRC Press, Boca Raton.

Hunt JL (2009) Loss of Heterozygosity, in *Cell and Tissue Based Molecular Pathology* (Tubbs RR and Stoler MH eds) pp 50-55, Churchill Livingstone, Philadelphia.

InnoGenomics Technologies L (2015) *Multi-Capillary IGT 5 Dye Matrix Standard ABI 3130 & 3500 Series Genetic Analyzers version 3.2*, InnoGenomics Technologies, LLC, New Orleans.

InnoGenomics Technologies L (2017) *InnoTyper® 21 Human DNA Analysis Kit – User Guide v3.8*, InnoGenomics Technologies, LLC, New Orleans.

InnoGenomics Technologies L (2018) *InnoQuant® HY-R Human and Male DNA Quantification & Degradation Assessment Kit for Use with non-7500 Instruments v 2.0*, InnoGenomics Technologies, LLC, New Orleans.

Kloosterman AD and Kersbergen P (2003) Efficacy and limits of genotyping low copy number (LCN) DNA samples by multiplex PCR of STR loci. *Journal of Social and Biological Systems* **197**:351-359.

Lagoa AM and Pinheiro MdF (2008) Amostras Low copy number, in *CSI Criminal* (Pessoa UF ed) pp 59-94, Universidade Fernando Pessoa, Porto, Portugal.

LaRue BL, Sinha SK, Montgomery AH, Thompson R, Klaskala L, Ge J, King J, Turnbough M and Budowle B (2012) INNULs: A novel design amplification strategy for retrotransposable elements for studying population variation. *Human Heredity* **74**:27-35.

LeFebvre AK, Ayers S, Gahn L, Sinha S, Brown H, Murphy G and Sinha SK (2017) Improving STR Profile Success Rates for Property Crime Specimens using InnoQuant®Human DNA Quantification & Degradation Assessment Kit. *Journal of Forensic Investigation* **5**:1-9.

Loftus A, Murphy G, Brown H, Montgomery A, Tabak J, Baus J, Carroll M, Green A, Sikka S and Sinha S (2017) Development and validation of InnoQuant HY-R, a system for quantitation and quality assessment of total human and male DNA using high copy targets. *Forensic Science International: Genetics* **29**:205-217.

Lowther K, Reynaga E, Anstead J and Cassidy BG (2016) *Validation of the InnoQuant™ DNA Quantification & Degradation Assessment kit and the InnoTyper™ 21 PCR Amplification Kit for Use on Forensic Samples*, DNA solutions, Oklahoma City.

Luo S, Valencia C, Zhang J, Lee N-C, Slone J, Gui B, Wang X, Li Z, Dell S, Brown J, Maris Chen S, Chien Y-H, Hwu W-L, Fan P-C, Wong L-J, Atwal P and Huang T (2018) Biparental Inheritance of Mitochondrial DNA in Humans. *Proceedings of the National Academy of Sciences* **115**:13039-13044.

Maceo AV (2011) Anatomy and Fisiology of Adult Fiction Ridge Skin, in *The Fingerprint Sourcebook (National Institute of Justice ed)*, Creatspace, USA.

Martins C, Ferreira PM, Carvalho R, Costa SC, Farinha C, Azevedo L, Amorim A and Oliveira M (2018) Internal validation of two new retrotransposons-based kits (InnoQuant HY-R and InnoTyper 21) at a forensic lab. *Forensic Science International* **283**:1-8.

Martins T and Carvalho R (2008) Conceitos Básicos de Genética com aplicação à investigação criminal, in *CSI Criminal* (Pessoa EUF ed) pp 83-94, Edições Universidade Fernando Pessoa, Porto, Portugal.

McKiernan HE and Danielson PB (2017) Chapter 21 - Molecular Diagnostic Applications in Forensic Science, in *Molecular Diagnostics (Third Edition)* (Patrinos GP, Ansorge W and Danielson PB eds) pp 371-394, Academic Press, United kingdom & United States.

Moura-Neto RS, Mello ICT, Silva R, Maette APC, Bottino CG, Woerner A, King J, Wendt F and Budowle B (2018) Evaluation of InnoTyper(R) 21 in a sample of Rio de Janeiro population as an alternative forensic panel. *International Journal of Legal Medicine* **132**:149-151.

Muñoz-López M and García-Pérez JL (2010) DNA transposons: nature and applications in genomics. *Current Genomics* **11**:115-128.

Panneerchelvam S and Norazmi MN (2003) Forensic DNA profiling and database. *The Malaysian Journal of Medical Sciences* **10**:20-26.

Parliament CotEU (2016) European Union General Data Protection Regulation, (Data PCfP ed), March 2018, European Union.

Parsons R, Bates L, Walton-Williams L, Fieldhouse S and Gwinnet C (2016) DNA from Fingerprints: Attempting Dual Recovery. *CSEye* **6**:8-17.

Patwari P and Lee RT (2008) Mechanical control of tissue morphogenesis. *Circulation Research* **103**:234-243.

Peixoto AS and Ramos AS (2010) Filmes Finos & Revelação de Impressões Digitais Latentes. *Ciência & Tecnologia dos Materiais* **22**:29-47.

Pineda GM, Montgomery AH, Thompson R, Indest B, Carroll M and Sinha SK (2014) Development and validation of InnoQuant™, a sensitive human DNA quantitation and degradation assessment method for forensic samples using high copy number mobile elements Alu and SVA. *Forensic Science International: Genetics* **13**:224-235.

Pinheiro MdFT (2008) A Pericia em Genética e Biologia Forense - Criminalística Biológica, in *CSI Criminal* (Pessoa UF ed) pp 11-40, Universidade Fernando Pessoa, Porto, Portugal.

Pinheiro MdFT (2010) Algumas perspectivas da identificação genética, in *Genética forense: perspectivas da identificação genética* (Pessoa UF ed) pp 17-137, Universidade Fernando Pessoa, Porto, Portugal.

Ramotowski R (2012) *Lee and Gaensslen's Advances in Fingerprint Technology, Third Edition*, CRC Press, United Kingdom.

República Ad (2009) Portaria n.º 270/2009, (República Ad ed) pp 1704 - 1704, Diário da República n.º 53/2009, Série I de 2009-03-17, Portugal.

República Ad (2017a) Lei n.º 90/2017, (República Ad ed) pp 4892 - 4906, Diário da República n.º 161/2017, Série I de 2017-08-22, Portugal.

República Ad (2017b) Lei nº67/2017, (República Ad ed) pp 4566 - 4570, Diário da República n.º 153/2017, Série I de 2017-08-09, Portugal.

Risoluti R, Filetti V, Iuliano G, Niola L, Schiavone S, Arcudi G and Materazzi S (2019) Updating procedures in forensic chemistry: One step cyanoacrylate method to develop latent fingermarks and subsequent DNA profiling. *Microchemical Journal* **147**:478-486.

Saferstein R (1998) *Criminalistics : an introduction to forensic science*, Prentice Hall, Upper Saddle River, New Jersey.

Santos AD (2019) Latent Fingermark Chemical Profiling: Identification of Time-dependent Metabolic Markers, in *Forensic Sciences and Laboratory Techniques*, Instituto Superior Ciências da Saúde, Gandra, Portugal.

Simas A and Calisto F (2001) *Lofoscopia*, SIJ/DCICPT, Lisboa.

Singh RS (2001) Polymorphism, in *Encyclopedia of Genetics* (Brenner S and Miller JH eds) pp 1507-1509, Academic Press, New York.

Singh Sankhla M and Kumar R (2017) Identification of Criminal by using Touch DNA: A new Tool for Investigation in Forensic Science. *Imperial Journal of Interdisciplinary Research (IJIR)* **3**:877-882.

Sinha S (2013) *Method for genetic detection using interspersed genetic elements: a multiplexed dna analysis system*, Google Patents, USA.

Sinha S, Murphy G, Brown H, Green A, Montgomery A, Carrol M and Tabak J (2015) Retrotransposable elements: Novel and sensitive DNA markers and their application in human identity. *Forensic Science International: Genetics Supplement Series* **5**:627-629.

Skinauthority (2019) The Healthy Skin Lifestyle Company, Retrieved 25/04/2019, http://www.skinauthority.com/Skin-Explained.

Snustad DP and Simmons MJ (2008) *Fundamentos de genética*, Guanabara Koogan, Rio de Janeiro.

Souto L (2010) Interesse dos microsatélites na identificação genética, in *Genética Forense - Perspectivas da identificação genética* (Pessoa UF ed) pp 79-102, Universidade Fernando Pessoa, Porto, Portugal.

StolenCars24 (2017) The European Database for Stolen Vehicles, Retrieved 14/04, 2019, https://www.stolencars24.eu, Germany.

Tao X, Chen X, Yang X and Tian J (2012) Fingerprint recognition with identical twin fingerprints. *PLoS One* **7**:35704-35704.

Trent RJ (2012) Chapter 9 - Forensic Science and Medicine, in *Molecular Medicine (Fourth Edition)* (Trent RJ ed) pp 275-299, Academic Press, Boston/Waltham.

Turss U, Turss R and Refojo MF (1970) Removal of Isobutyl Cyanoacrylate Adhesive from the Cornea with Acetone. *American Journal of Ophthalmology* **70**:725-728.

van Dam A, Schwarz JC, de Vos J, Siebes M, Sijen T, van Leeuwen TG, Aalders MC and Lambrechts SA (2014) Oxidation monitoring by fluorescence spectroscopy reveals the age of fingermarks. *Angewandte Chemie (International ed in English)* **53**:6272-6275.

van Oorschot RA and Jones MK (1997) DNA fingerprints from fingerprints. *Nature* **387**:767.

Vilar MFS (2015) Identificação de Potenciais Variações Genéticas na Diversidade Individual das Impressões Digitais, Instituto de Ciências Biomédicas Abel Salazar na Universidade do Porto, Porto, Portugal.

von Wurmb N, Meissner D and Wegener R (2001) Influence of cyanoacrylate on the efficiency of forensic PCRs. *Forensic Science International* **124**:11-16.

Yamashita B and French M (2011) Latent Print Development, in *The Fingerprint Sourcebook (National Institute of Justice ed)* (Programs USDoJOoJ ed), Creatspace.

Attachment 1 – Volunteer´s identification sheet.

CESPU
INSTITUTO UNIVERSITÁRIO DE CIÊNCIAS DA SAÚDE

QUESTIONÁRIO AO VOLUNTÁRIO

Identificação

Nome: _____

Idade: ____ anos Sexo: F ☐ M ☐ Profissão: _____

Contacto telefónico: _____ E-mail: _____

Código de Identificação do Voluntário (a preencher pelo investigador principal): ☐

Questionário

A participação neste projeto de investigação é voluntária. Apenas o investigador principal terá acesso às informações pessoais, sendo o preenchimento deste inquérito totalmente confidencial.

De seguida encontra-se um conjunto de questões pertinentes para o estudo. Deve ler atentamente as questões e ser o mais fidedigno possível nas suas respostas. Estas devem traduzir a realidade dos factos questionados. Não existem respostas certas ou erradas.

Em cada questão, assinale com um (X) a resposta que melhor traduzir a sua experiência.

Selecione apenas uma opção por questão, em caso de rasura, assinalar a opção selecionada de forma inequívoca.

1. Normalmente, quantas vezes lava as mãos por dia?
   - ☐ Não Lava
   - ☐ Entre 1-3 vezes
   - ☐ Entre 4-6 vezes
   - ☐ Mais de 10 vezes

   Se respondeu "Não Lava", justifique a razão:
   - ☐ Esquecimento
   - ☐ Não é rotina
   - ☐ É uma perda de tempo
   - ☐ Outras razões: _____

2. Se respondeu afirmativamente à questão nº 1, o que utiliza usualmente para lavar as mãos?
   - ☐ Só água
   - ☐ Água e sabão
   - ☐ Água e detergente da loiça
   - ☐ Água e gel desinfetante
   - ☐ Outros: _____

3. Se respondeu afirmativamente à questão nº 1, na maioria das vezes seca as mãos depois de lavar?
   - ☐ Sim, com papel
   - ☐ Sim, com o secador elétrico
   - ☐ Sim, com uma toalha
   - ☐ Não

4. Se respondeu afirmativamente à questão nº 1, após lavar as mãos realiza higienização antisséptica com álcool?
   - ☐ Sim
   - ☐ Não
   - ☐ Às vezes

5. Se respondeu afirmativamente à questão nº 1, realiza a seguinte técnica de lavagem de mãos?

Fonte: adaptado de http://congregar.acsc.org.br/wp-content/uploads/2013/08/Lavar-as-m%C3%A3os1.jpg

   - ☐ Sim
   - ☐ Não
   - ☐ Às vezes

6. Costuma aplicar creme nas mãos?
   - ☐ Sim
   - ☐ Não
   - ☐ Às vezes

7. Como classifica a sua sudação nas mãos?
   - ☐ Quase inexistente
   - ☐ Normal
   - ☐ Excessiva

8. Tem por hábito roer as unhas e/ ou a pele em torno das unhas?
   - ☐ Sim
   - ☐ Não
   - ☐ Às vezes

9. Refira qual o número que melhor representa a forma como segura o volante automóvel enquanto conduz: _____

Fonte: adaptado de http://www.rondoniaovivo.com/imagens_editor/522.jpg

Muito obrigada.

_____

(Assinatura do voluntário)

CESPU
INSTITUTO UNIVERSITÁRIO
DE CIÊNCIAS DA SAÚDE

Consentimento Informado para Tratamento dos Dados Lofoscópicos e Genéticos

Designação do Estudo: "**Vestígios humanos em superfícies internas de carros: Ampliando as aplicações da análise genética**"

Os crimes referentes a assaltos a veículos automóveis e realização de carjacking têm vindo a aumentar nos últimos anos, sendo uma necessidade urgente efetuar uma correta identificação dos indivíduos envolvidos em tais eventos. Alguns tipos de superfícies internas dos carros, tal como a pele sintética existente em muitos volantes automóveis, impõem normalmente dificuldades adicionais na análise de impressões digitais. Desta forma, o presente projeto tem como principal objetivo abordar essas dificuldades, testando a compatibilidade e complementaridade da análise de impressões digitais com a análise de ADN, a fim de alcançar com sucesso a identificação humana de quem comete atos ilícitos neste contexto. Pretende-se avaliar a possibilidade destas duas áreas científicas serem ferramentas sinergísticas e promissoras num cenário forense que envolva veículos automóveis, contribuindo para o desenvolvimento de novos protocolos de atuação, face a cenários reais.

Eu, _____ (nome completo do voluntário), declaro que compreendi a explicação que me foi fornecida acerca da participação no presente projeto de investigação (objetivos, métodos, riscos e benefícios) e autorizo, de livre vontade, que os meus dados lofoscópicos (impressões digitais) e genéticos (perfil genético) sejam usados neste estudo, exclusivamente. Afirmo que, inclusivamente, me foi comunicado que findo o presente estudo, o questionário ao voluntário será destruído, deixando de existir a possibilidade de estabelecer uma correspondência entre o voluntário em questão e os seus dados lofoscópicos e genéticos. Declaro ainda que solicitei as informações de que necessitei, sabendo que o esclarecimento é fundamental para a tomada de uma boa decisão.

Concordo em participar no estudo suprarreferido, de acordo com os esclarecimentos que me foram prestados e, por tal ser verdade, assino abaixo.

Data ____/____/_____

_____

(Assinatura do voluntário)

CESPU
INSTITUTO UNIVERSITÁRIO
DE CIÊNCIAS DA SAÚDE

Comissão de Ética
Instituto Universitário de Ciências da Saúde
Contacto: 224 157 136
E-mail: carla.ribeiro@cespu.pt

CARTA RESPOSTA

Título do projeto: Vestígios humanos em superfícies internas de carros: ampliando as aplicações da análise genética.
Investigador responsável: Áurea Marília Madureira e Carvalho
Nº Registo: 7/CE-IUCS/2017

Parecer:

Exmo(a). Senhor(a),

Em resposta ao pedido efectuado por V. Exa. a esta Comissão de Ética, para emissão de parecer sobre o projecto de investigação supra identificado, somos a informar que, e de acordo com o regulamento, o mesmo recebeu parecer favorável por parte desta Comissão.

Gandra, 2[illegible] fevereiro de 2018

CESPU
INSTITUTO UNIVERSITÁRIO
DE CIÊNCIAS DA SAÚDE
R. Central de Gandra, 1317
T. +351 224157100 • F. +351 224157181
Comissão de Ética

Prof. Doutor Jorge Brandão Teixeira
Presidente da Comissão de Ética

Volunteer´s numerical identification	Database/ Reference lophoscopic bulletins	Database/ Reference genetic samples	Vestigial/ Questioned samples without cyanoacrylate	Steering wheels + Vestigial/ Questioned samples developed with cyanoacrylate
1	J	K	K	L
2	C	I	D	H
3	H	G	C	B
4	A	H	A	E
5	D	D	L	D
6	F	E	J	J
7	G	C	I	C
8	E	F	B	G
9	I	B	F	A
10	K	J	E	F
11	B	A	H	I
12	L	L	G	K

CESPU INSTITUTO UNIVERSITÁRIO DE CIÊNCIAS DA SAÚDE Dissertação "Human Traces from car inner surfaces: Broadening the application of genetic policing"			Fórmula decadactilar — — — — — - — — — —		
			Fórmula palmar — — — - — — —		
mão direita	1.polegar	2.indicador	3. médio	4. anelar	5. auricular
mão esquerda	6.polegar	7.indicador	8.médio	9.anelar	10.auricular

CESPU INSTITUTO UNIVERSITÁRIO DE CIÊNCIAS DA SAÚDE Dissertação "Human Traces from car inner surfaces: Broadening the application of genetic policing"		Código de identificação do voluntário ☐		
	mão esquerda	polegar	polegar	mão direita
mão direita				
mão esquerda				

Attachment 6 - Quiroscopic bulletin model.