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Laura Sofia Ramos Mendes Cainé

Y-STR markers, haplotype discrimination and sensibility studies in sexual assault cases – The impact of different technology

março, 2016

FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO

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Y-STR markers, haplotype discrimination and sensibility studies in sexual assault cases – The impact of different technology

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FMUP FACULDADE DE MEDICINA UNIVERSIDADE DO PORTO
UNIDADE CURRICULAR PROJETO DE OPÇÃO DISSERTAÇÃO / MONOGRAFIA

Projeto de Opção do 6º ano - DECLARAÇÃO DE INTEGRIDADE

Eu, ____Laura Sofia Ramos Mendes Cainé_____, abaixo assinado, nº mecanográfico _200805533_____, estudante do 6º ano do Ciclo de Estudos Integrado em Medicina, na Faculdade de Medicina da Universidade do Porto, declaro ter atuado com absoluta integridade na elaboração deste projeto de opção.

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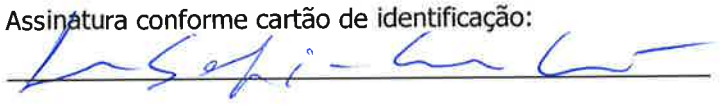

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Projecto de Opção do 6º ano – DECLARAÇÃO DE REPRODUÇÃO

NOME

Laura Sofia Ramos Mendes Cainé

NÚMERO DE ESTUDANTE	DATA DE CONCLUSÃO
200805533	2016

DESIGNAÇÃO DA ÁREA DO PROJECTO

Crimes sexuais

TÍTULO DISSERTAÇÃO/MONOGRAFIA (riscar o que não interessa)

Y-STR markers, haplotype discrimination and sensibility studies in sexual assault cases - The impact of different technology.

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Ao meu António e ao meu Martim Maria.

Title: Y-STR markers, haplotype discrimination and sensibility studies in sexual assault cases – The impact of different technology

Authores: #Benedita Silva[1], #Laura Cainé[1,2,3], Maria João Porto[2,3], Teresa Magalhães[1,2]

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Abstract

Sexual assault biological evidence are among the most difficult samples types encountered in forensic laboratories. Y-STR markers constitute a valuable tool in the analysis of these traces. Yfiler™ Plus PCR Amplification Kit (Applied Biosystems) and AmpFLSTR® Yfiler® PCR Amplification Kit (Applied Biosystems) constitute two different technologies that can be used on this analysis. The aim of this project is to compare the success rate of these two kits. A total of 325 trace samples were quantified and analyzed. Yfiler™ Plus PCR Amplification Kit presented a greater robustness and sensitivity, especially on samples with higher female:male DNA ratio. This kit presented better results in 43% of all samples compared to the 5% obtained with AmpFLSTR® Yfiler® PCR Amplification Kit. Furthermore, Yfiler®Plus PCR Amplification Kit amplified a higher number of samples than AmpFLSTR® Yfiler® PCR Amplification Kit (127 and 97, respectively). It was concluded that Yfiler™ Plus PCR Amplification Kit constitutes an excellent alternative to AmpFLSTR® Yfiler® PCR Amplification Kit and a good option to implement in laboratories.

Introduction

Biological evidence resulting from sexual assault perpetrated by males are among the most difficult samples for forensic laboratories to deal with [1-3]. Typically this kind of samples present multiple challenges including small quantity of male DNA, relatively high quantity of victim DNA, the presence of PCR inhibitors, contamination or degradation [4-6]. This often results in failure to obtain an autosomal STR profile from the perpetrator DNA [7]. Therefore, there is a need for robust and highly sensitive methods to genotype these samples.

Y-STR markers are a valuable tool in the analysis of these biological traces [8-9]. It is possible to generate a Y-chromosome DNA profile, even if all the prior semen tests are negative [10]**.** Nowadays, some Y-STR specific kits are being improved to promote obtaining complete profiles - namely Yfiler™ Plus PCR Amplification Kit [9,11] - Yfiler® Plus (Applied Biosystems) and AmpFLSTR® Yfiler® PCR Amplification Kit - Yfiler® (Applied Biosystems). The Yfiler®Plus kit is an expanded and improved version of Yfiler® kit, which simultaneously targets 27 markers on the Y chromosome (*e.g.* 25 markers, in which two of them is referred to heterozygote markers), while Yfiler® only targets 17 markers on Y chromosome (one of them is a heterozygote marker). The higher the number of *loci* analyzed, the higher is the discriminative power of the kit used [9].

The aim of this study is to compare the two different technologies of Y-STR amplification used in routine forensic casework of sexual assault trace samples, in order to assess the impact on the success rate in this case.

Materials and methods

Samples characteristics

A total of 325 trace samples were amplified and analyzed with both Yfiler®Plus kit and Yfiler® kit as part of routine casework (sexual crimes).

Laboratory techniques

Casework samples were extracted with PrepFiler® Express Forensic DNA Extraction Kit (Applied Biosystems), on the AutoMate Express™ Forensic DNA Extraction System (Applied Biosystems).

All the samples were previously quantified (Quantifiler®Trio DNA quantification kit), in 7500 Real-Time PCR System (Applied Biosystems), which provided female:male DNA ratio and the specific Y chromosome quantity. Samples presented varying amounts of male DNA: bellow 0,001ng (most of the samples) and above 30ng.

The amplification with both commercial kits occurred using half of the amount recommended by the manufacturers, and this procedure were previously validated in the laboratory promoting valid and robust results. Considering this alteration to the manufacturers' protocol, samples that presented quantities above 4ng were thereafter diluted to ideal quantities (approximately 1ng) and it was used 1µL of sample. On the other hand, samples that presented quantities bellow 0,5ng were amplified with the maximum sample allowed by the manufacturers (it was used 5µL of sample).

All samples were amplified and analyzed using the Yfiler® and Yfiler®Plus kits. They amplify 17 and 27 Y-STR *loci*, respectively, in a single polymerase chain reaction (PCR). The first kit amplifies the *loci* included in the, *European*

Minimal Haplotype (DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393), in the *Scientific Working Group-DNA Analysis Methods (SWGDAM)-recommended Y-STR panel* (European Minimal Haplotype plus DYS438 and DYS439) and additional highly polymorphic *loci* (DYS437, DYS448, DYS456, DYS458, DYS635 (Y GATA C4) and Y GATA H4). The Yfiler®Plus kit amplifies the 17 *loci* contained in the Yfiler® kit and 10 extra *loci* (DYS576, DYS627, DYS460, DYS518, DYS570, DYS449, DYS481, DYF387S1a/b, DYS533).

DNA amplification, fragment separation and analysis were performed according to the manufacturer's recommendations for the referred kits. This investigation stages were performed on an Applied Biosystems® GeneAmp® PCR System 9700 (thermocycler), an Applied Biosystems® 3500 Genetic Analyzer, and a GeneMapper® Software, respectively.

Contamination issues

All the procedures (extraction, amplification and fragment analysis) were performed only by female experts to avoid eventual secondary Y-DNA contamination.

Ethical considerations

The present project was approved by the Ethical Commission of Health of the Hospital de São João and by the Investigation, Formation and Documentation Department of National Institute of Legal Medicine and Forensic Sciences.

Full anonymity was secured in laboratory and statistical analysis.

Statistical analysis

The results obtained were then compiled, analyzed and compared recurring to IBM SPSS Statistics (software version 23.0). Only when the minimum haplotype was totally amplified, we considered the obtained results valid and presenting the necessary minimum information for a possible suspect's identification. The minimum haplotype is constituted by 9 *loci* (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385a/b), which still forms the core of all Y-STR kits in current forensic use [12].

All data compilation was performed on SPSS program. Results were presented as percentages, which were calculated manually in the same program. To verify if there were significant differences in amplification performance between the two commercial kits, a "Paired Sample t test" was performed on SPSS.

Results and Discussion

Using results obtained from real trace samples collected within sexual crimes investigation has the advantage of allowing us to understand the real difficulties found during this type of investigations and observe the real advantages of using a specific DNA analysis amplification kit.

The improvement of Yfiler®Plus kit reagents, compared to Yfiler® kit, promote a better performance of the kit, as showed in Table 1.

Table 1 – Comparative results obtained with the two kits used.

	Yfiler®	**Yfiler®Plus**
Total Amplified Samples - minimum haplotype	97 (29,8%)	127 (39,1%)
Total Amplified Samples – more informative haplotype	16 (4,9%)	139 (42,8%)

Yfiler®Plus kit presented a more informative haplotype (*e.g.* a higher number of *loci* amplified and a better amplification performance) in 43% (n=139) of all samples (n=325), while Yfiler® kit only showed that in 5% (n=16) of the samples. Considering minimum haplotype, Yfiler®Plus kit amplified successfully a greater number of samples. In total, 127 samples (39,1%) were successfully amplified with Yfiler®Plus kit, while only 97 samples (29,8%) were successfully amplified with Yfiler® kit.

Yfiler®Plus kit revealed greater robustness and sensitivity than Yfiler® kit, especially in samples with high proportions of female DNA. In five of the analyzed samples, presenting male DNA quantities bellow 0,1ng/µL and female DNA quantities above 1ng/µL, Yfiler®Plus kit allowed us to realize that the DYS385a/b were heterozygote for that samples, instead of homozygote as obtained by Yfiler® kit.

With the compilation of this data, we were able to notice that in different samples, referring to different cases, the haplotype obtained by Yfiler® kit was the same, preventing us from distinguishing them. When the same samples were typed with Yfiler® Plus kit, we could differentiate them. Those extra *loci* amplified in Yfiler® Plus kit were extremely important for this differentiation, since it gave us extra important genetic information. This way, Yfiler® Plus kit revealed a higher discriminative power than Yfiler® kit. This study of the difference of discriminative power between the two kits could be also done recurring to reference samples of the suspects of the different cases, but, unfortunately, in most cases, we do not have a reference sample of a suspect to compare.

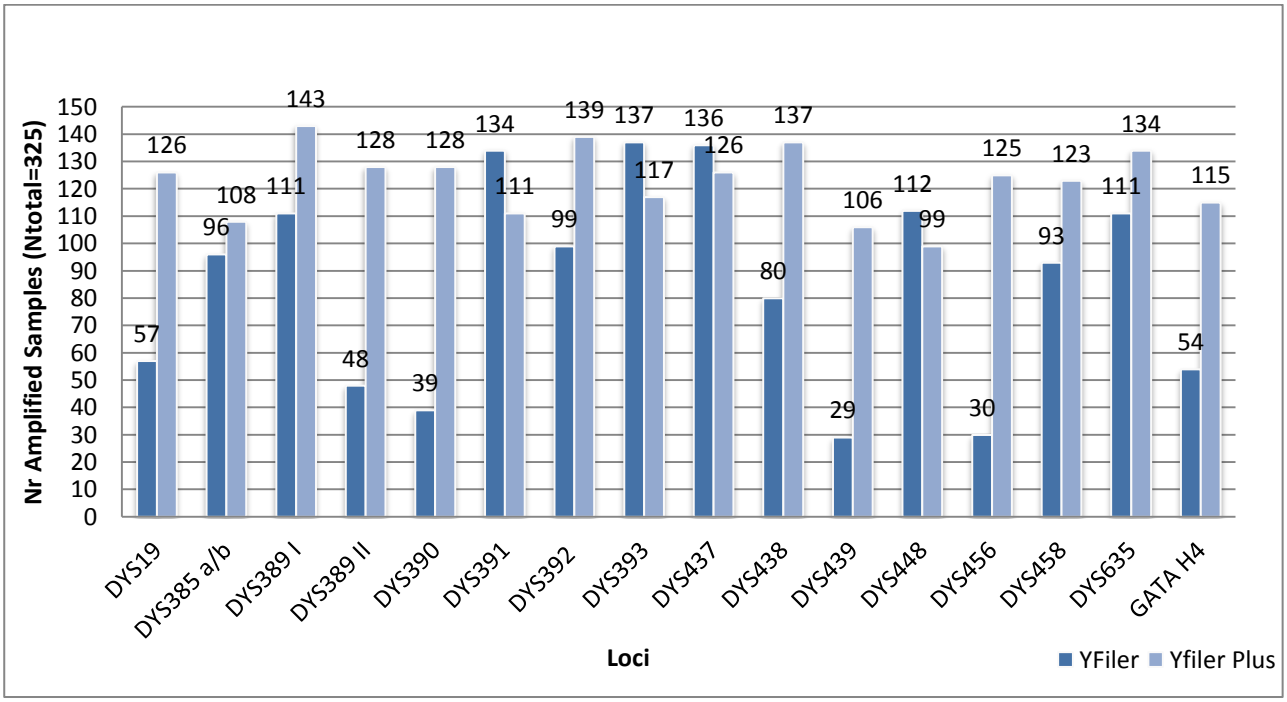

Figure 1 – Graphic presenting the comparison between Yfiler®Plus and Yfiler®, concerning the amplification of the different *loci*.

In Figure 1 it can be observed that in most *loci* Yfiler®Plus kit presented a better amplification performance, except in DYS391, DYS393, DYS437 and DYS448 *loci*.

The “Paired Sample t test” (Figure 2) allowed us to verify that there were significant differences in the amplification performance between the Yfiler®Plus kit and Yfiler® kit (α=0,05; p=0,002).

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	VAR00001 - VAR00002	37,43750	39,97994	9,99499	16,13369	58,74131	3,746	15	,002

Figure 2 – Results obtained with SPPS “Paired Sample t test”. VAR00001 – YFiler®Plus; VAR00002 – Yfiler®.

In 52,3% of all samples (n=170) it was impossible to obtain an Y-STR profile for both kits. This can be explained by the fact that we are analyzing real trace samples, which are associated with degradation, contamination (bacteriological, fungi) and, besides that, it is sometimes also associated with a poor and barely conducted collection, storage and preservation of the sample [1]. Not always in this type of samples we are able to found the perpetrator contribution (semen, saliva or other biological sample).

For Yfiler®Plus kit, we started to observe failures in the amplification on samples in which male DNA quantities of most of these were below 0,02ng/µL, while in Yfiler® kit these failures have begun to be observed in samples presenting quantities approximately 0,8ng/µL. This way, we may conclude that Yfiler®Plus kit present higher sensitivity.

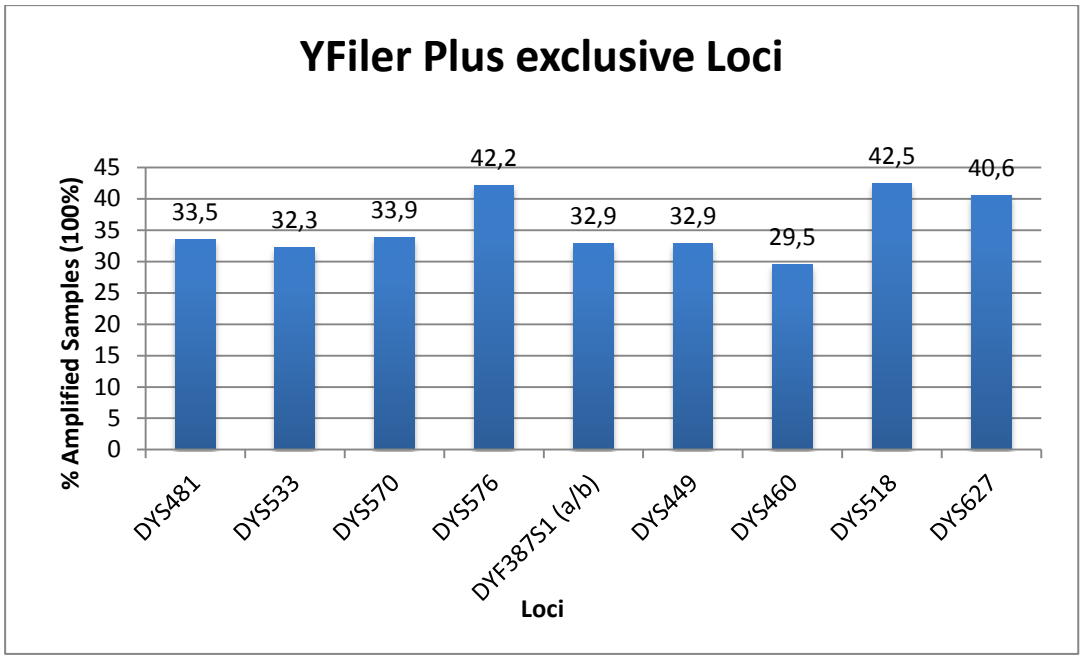

Figure 3 – Graphic presenting the results concerning the amplification of specific Yfiler®Plus *loci*.

In Figure 3 the percentage of amplified samples for the exclusive Yfiler®Plus kit *loci* can be observed. For approximately 40% of the samples this kit promoted extra information compared to Yfiler® kit, allowing a higher discrimination power.

Conclusions

Yfiler®Plus kit was found to be more robust, sensitive, sensible and more discriminative than Yfiler®kit. The improvement of Yfiler®Plus kit reagents led to a more successful amplification of Y-STR alleles compared to Yfiler® kit. This, consequently, promoted a higher number of samples successfully amplified (39%) and a significant number of samples with higher amplification performance (43%), even in samples with low quantity of Y chromosome and high female:male DNA ratio.

Due to the previous statements, it was concluded that the overall success of Y-STR analysis with Yfiler®Plus kit makes it an excellent alternative to Yfiler® kit, and its implementation on laboratories may be a good option.

So it is very important to be alert to the upgrading and improvement of the technology, to increasingly achieve better results.

Conflict of interest

None

Funding

None

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À Senhora Professora Doutora **Teresa Magalhães**, orientadora deste trabalho, certa de não dispor de palavras suficientes para exprimir a minha gratidão por toda a sua orientação, sensatez e rigor com que sempre me aconselhou.

À Senhora Dra. **Maria João Porto**, Directora do Serviço de Genética e Biologia Forenses do Instituto Nacional de Medicina Legal e Ciências Forenses, IP, o meu profundo reconhecimento pelo apoio, disponibilidade e excelente espírito crítico ao longo de todo este trabalho.

À **Benedita Ferreira**, colega e amiga, pela indispensável colaboração na redacção escrita deste estudo, pelo carinho e amizade, imprescindíveis para a prossecução deste trabalho.

A todos os meus colegas pela paciência e pelo bom ambiente de trabalho que me proporcionaram.

Ao meu marido **António**, por estar presente com toda a sua compreensão, em especial nas horas em que tudo parecia esmorecer e ao meu filho **Martim Maria**, simplesmente por existir.

ANEXOS

SÃO JOÃO
HOSPITAL

Comissão de Ética para a Saúde do HSJ

Parecer

Projeto intitulado "Y-STR markers, haplotype discrimination and sensibility in sexual assault cases. The impact of different technologies"

Estudo que pretende vir a ser desenvolvido no Serviço de Genética do Instituto Nacional de Medicina Legal e Ciências Forenses (IMLCF) da UP para efeitos de elaboração de tese de mestrado integrado em medicina pela aluna da FMUP Laura Sofia Ramos Mendes Cainé, sob orientação da profª Teresa Magalhães.

Do ponto de vista científico trata-se de um estudo cujo objectivo principal consiste em comparar os resultados obtidos com três diferentes metodologias de amplificação utilizadas para a obtenção de perfis genéticos, no âmbito de perícias criminais de natureza sexual, com diferentes kits. Todas as amostras foram previamente quantificadas pelos referidos 3 métodos que irão ser comparados.

Está garantida pela directora do Serviço de Genética, a privacidade sobre a informação relativa às amostras estudadas

Não está previsto o acesso a dados clínicos nem uso de questionários.

Face à natureza do estudo o consentimento informado é dispensável.

O estudo está autorizado pelo director do IMLCF da UP e pela directora do Serviço de Genética do IMLCF da UP.

Em face da análise do protocolo proponho a sua aprovação pela CES do CHSJ.

Porto, 23 de Setembro de 2015

O relator

Prof. Manuel Pestana

7. SEGURO

a. *Este estudo/projecto de investigação prevê intervenção clínica que implique a existência de um seguro para os participantes?*

SIM ☐ (Se sim, junte, por favor, cópia da Apólice de Seguro respectiva)

NÃO ☒

NÃO APLICÁVEL ☒

8. TERMO DE RESPONSABILIDADE

Eu, LAURA SOFIA RAMOS MENDES CAIRÉ,

abaixo-assinado, na qualidade de Investigador Principal, declaro por minha honra que as informações prestadas neste questionário são verdadeiras. Mais declaro que, durante o estudo, serão respeitadas as recomendações constantes da Declaração de Helsínquia (com as emendas de Tóquio 1975, Veneza 1983, Hong-Kong 1989, Somerset West 1996 e Edimburgo 2000) e da Organização Mundial da Saúde, no que se refere à experimentação que envolve seres humanos. Aceito, também, a recomendação da CES de que o recrutamento para este estudo se fará junto de doentes que não tenham participado em outro estudo no decurso do actual internamento ou da mesma consulta.

Porto, 04 / SETEMBRO / 2015

O Investigador Principal

PARECER DA COMISSÃO DE ÉTICA PARA A SAÚDE DO CENTRO HOSPITALAR DE S. JOÃO/FACULDADE DE MEDICINA DA UNIVERSIDADE DO PORTO

emitido na reunião plenária da CES de 25 / Setembro / 2015

A Comissão de Ética para a Saúde APROVA por unanimidade o parecer do Relator, pelo que nada tem a opor à realização deste projecto de investigação.

Prof. Doutor Filipe Almeida
Presidente da Comissão de Ética

Exma. Senhora Diretora do Departamento de Investigação, Formação e Documentação do Instituto Nacional de Medicina Legal e Ciências Forenses, I.P.

DESPACHO DO RESPONSÁVEL PELO ACESSO À INFORMAÇÃO

ASSUNTO: **Pedido para utilização, no âmbito de trabalho científico a apresentar em Mestrado Integrado da Faculdade de Medicina da Universidade do Porto. Título do projeto/artigo: «*Y-STR markers, haplotype discrimination and sensibility studies in sexual assault cases. The impact of different technologies*». Requerimento de 7-4-2015. Elemento que vai desenvolver o projeto no INMLCF: Laura Cainé (especialista superior de medicina legal do Serviço de Genética e Biologia Forenses do INMLCF); Responsáveis pelo desenvolvimento do projeto no INMLCF: Professora Doutora Teresa Magalhães, e Dra. Maria João Porto.**

A) **CONSIDERANDO:**

1. O estudo que vem descrito na "ficha-resumo de projetos de investigação científica ou de artigos científicos, a decorrer ou a desenvolver", insere-se na atividade científica do INMLCF, que é sua expressa atribuição legal (alínea *c* do n.º 2 do artigo 3.º do Decreto-Lei n.º 166/2012, de 31 de julho)[1], atividade que se revela fundamental para a constante atualização do conhecimento científico que diretamente constitui óbvio suporte da continuidade e da melhoria da qualidade das perícias médico-legais e forenses e, em geral, da intervenção pericial dos serviços médico-legais.

2. Nos termos do n.º 1 do artigo 20.º Decreto-Lei n.º 166/20012, de 31 de julho, «o INMLCF, I.P., prossegue as suas atribuições e exerce as suas competências em colaboração com os estabelecimentos de ensino superior, especialmente escolas médicas, nomeadamente de investigação, públicas ou privadas, mediante a celebração de protocolos nas áreas do ensino, da formação e da investigação científica.». Presume-se, pois, que a atividade que se pretende realizar recebe o devido enquadramento institucional justificador da colaboração com a Faculdade de Medicina da Universidade do Porto.

3. O estudo a realizar tem por objetivo a comparação de três diferentes tecnologias de amplificação de Y-STRs, necessária para uma resposta mais eficaz nas perícias, designadamente nas que são realizadas a partir de amostras resultantes de agressões sexuais. O estudo terá, pois, impacto direto e positivo na atividade pericial, proporcionando o reforço da qualidade da intervenção pericial daquele Serviço do INMLCF, pelo que é do seu manifesto interesse.

[1] É atribuição do INMLCF, nos termos desta norma, «desenvolver atividades de investigação e divulgação científicas, de formação e de ensino, no âmbito da medicina legal e de outras ciências forenses e desenvolver formas de colaboração científica e pedagógica com outras instituições».

4. A utilização, nos termos constantes do resumo do projeto, das amostras colhidas para efeitos periciais, constitui um procedimento de boas práticas periciais, porquanto visa a permanente atualização técnica e científica da resposta que ao SGBF cumpre dar às solicitações das autoridades judiciárias e judiciais.

B) **PRESSUPONDO:**

1. O estudo a realizar não prejudicou, nem prejudicará, a eventual utilização das amostras nos termos e para os efeitos do disposto no artigo 25.º da Lei n.º 45/2004, de 19 de agosto (este Artigo define o regime de recolha, depósito, conservação e destruição de produtos biológicos recolhidos no âmbito de perícias médico-legais e forenses).[2]

2. Antes da utilização laboratorial das amostras para os fins do estudo procede(u)-se à anonimização irreversível das mesmas, do que resulta a anonimização irreversível dos perfis de ADN.

3. O estudo não implica o acesso a informação de natureza pericial relativa às amostras, ou aos processos médico-legais nos quais estas se integram.

C) **DECIDINDO:**

1. Inexistem indícios de que a atividade proposta colida com outras responsabilidades, designadamente periciais ou processuais, deste Instituto.

2. Pelo que, nos pressupostos e condições acima assinalados em A) e B), se confere **DEFERIMENTO** ao pedido.

26 de maio de 2015

O Responsável pelo Acesso à Informação

(Diogo Pinto da Costa)

[2] O mencionado artigo 25.º, n.º 1, sobre os exames periciais de recolha de produtos biológicos, determina expressamente que *após a realização do exame se proceda à recolha, acondicionamento e selagem de uma amostra suscetível de possibilitar a realização de nova perícia e à destruição do remanescente*. Mais determina o n.º 2 do mesmo artigo 25.º que *a amostra fica depositada no serviço médico-legal durante o período de dois anos, após o qual o serviço médico-legal pode proceder à sua destruição, salvo se, entretanto, o tribunal tiver comunicado determinação em contrário*.

FORENSIC SCIENCE INTERNATIONAL: GENETICS

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DESCRIPTION

Forensic Science International: Genetics is specifically devoted to **Forensic Genetics**. This branch of **Forensic Science** can be defined as the application of **genetics** to human and non-human material (in the sense of a science with the purpose of studying inherited characteristics for the analysis of inter- and intra-specific variations in populations) for the resolution of legal conflicts.The scope of the journal includes:Forensic applications of human polymorphism.Testing of paternity and other family relationships, immigration cases, typing of biological stains and tissues from criminal casework, identification of human remains by DNA testing methodologies.Description of human polymorphisms of forensic interest, with special interest in DNA polymorphisms.Autosomal DNA polymorphisms, mini- and microsatellites (or short tandem repeats, STRs), single nucleotide polymorphisms (SNPs), X and Y chromosome polymorphisms, mtDNA polymorphisms, and any other type of DNA variation with potential forensic applications.Non-human DNA polymorphisms for crime scene investigation.Population genetics of human polymorphisms of forensic interest.Population data, especially from DNA polymorphisms of interest for the solution of forensic problems.DNA typing methodologies and strategies.Biostatistical methods in forensic genetics.Evaluation of DNA evidence in forensic problems (such as paternity or immigration cases, criminal casework, identification), classical and new statistical approaches.Standards in forensic genetics.Recommendations of regulatory bodies concerning methods, markers, interpretation or strategies or proposals for procedural or technical standards.Quality control.Quality control and quality assurance strategies, proficiency testing for DNA typing methodologies.Criminal DNA databases.Technical, legal and statistical issues.General ethical and legal issues related to forensic genetics

AUDIENCE

Forensic geneticists, forensic practioners in a broad sense, anthropologists, geneticists, lawyers, people interest in legal and ethical issues related with forensic genetics, mathematicians and statisticians interested in forensic genetic issues

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INTRODUCTION

Types of paper

1. Original Research Papers (Regular Papers)
2. Review Articles
3. Letters to the Editor
4. Case Reports
5. Book Reviews
6. Forensic Population Genetics (Original Paper, Short Communication or Letter to the Editor)
7. Rapid Communications

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