"RECCOMMENDATIONS FOR THE CORRECT USE OF DNA ANALYSIS WITH FORENSIC PURPOSES"

WORKING GROUP FIDE-FUNDACION GARRIGUES

Madrid, March 19th, 2019.

1- Presentation:

The Working Group created by **Fundación Fide** and **Fundación Garrigues** met in January and June 2018, in two working sessions, in which the following issues, among others, were analysed:

- **DNA and forensic sciences;**
- **STR Profiles and DNA Databases;**
- **Likelihood Ratio;**
- **Background DNA and primary and secondary DNA transfers;**
- **DNA contamination;**
- **Implementation of new massively parallel sequencing techniques and new DNA markers;**
- **Forensic science research.**

The main objective with which this Working Group has been convened, from civil society, **is to elaborate a series of recommendations for the correct use of DNA analysis for forensic purposes, stating the value of forensic evidence and the limit of its use.** The eight recommendations offered in this document are the result of the work carried out.

The conclusions contained in the document were drawn from the contributions and interventions of all the participants in the Group. Although, logically, they do not represent the unanimous opinion of all the members of the group, notably on the root causes of some of the current problems and their solutions, they do reflect the issues on which the debate and collective reflection focused.

2- Issues subject to analysis and recommendations.

1- DNA and forensic sciences:

DNA has become the most advanced, accurate and effective human identification forensic system of all forensic sciences.

Since the discovery of the so-called "genetic fingerprinting" by Alec Jeffreys' group, there has been an enormous evolution in technologies and types of DNA polymorphisms (known in Spain as identifiers) that are used to advise judges and courts and to help justice in civil and criminal cases, ranging from paternity tests, to the identification of individuals (alive or dead) and the analysis of biological traces of criminal interest in all types of crimes and not only against people, such as illegal trafficking in animals or products in which there is biological material subject to analysis.

The most commonly used DNA technology in forensic applications is based on **the analysis of the variability of short regions repeated in tandem** (Short Tandem Repeats: STRs) of our nuclear DNA, which has acquired enormous discriminatory power and also a high degree of

technical standardization worldwide, which has allowed for **an extensive exchange of DNA data between countries and a very demanding quality control and accreditation of laboratory.** Occasionally, mitochondrial DNA is also used, a type of DNA that is inherited through the mother (especially in the analysis of hair) and variations in single nucleotides (SNPs and InDels) very useful in the case of degraded DNA.

Generally attempts have been made to choose polymorphisms in non-coding DNA regions with the intention of making the analysis as least informative as possible but this has led to the misconception that non-coding DNA analysis is completely neutral in terms of information unrelated to the purpose of identification. This is not true. **Non-coding and coding DNA are not synonymous with functionality.** There are variations in coding DNA that are not informative and functional variations in non-coding DNA that is where DNA regulation is found. From the analysis of forensic DNA it is possible, besides knowing the sex of the person, to suspect if he/she suffers from some chromosomopathies such as Down syndrome and especially alterations in sexual chromosomes, as well as if he/she suffered an allogeneic bone marrow transplant. Eventually, anomalies could be found in the DNA profiles as a consequence of cancers with micro-satellite instability, such as some forms of colorectal cancer and leukaemias.

1st Recommendation:

Forensic DNA technology is completely valid and effective, but the information derived from the analysis is not absolutely neutral in terms of information related to the health or condition of the individual.

The erroneous idea that non-coding DNA is not informative in terms of information additional to that which is merely identifying in some cases (trisomies, linking variants to diseases, etc.) should be banished from legislation and doctrine.

In any case, the use of forensic DNA for identification purposes in criminal cases is considered to be proportionate, even though it may provide some sensitive information on the subject.

2- STR Profiles and DNA Databases:

DNA technology based on the analysis of Short Tandem Repeats (STRs) regions has become the method of choice and the most widely used international standard of DNA analysis in thousands of forensic genetics laboratories around the world. The development of national databases of STR profiles has enabled systematic searches of DNA profiles (beyond DNA profile searches in a specific case) providing a new DNA analysis strategy for the identification of persons in the investigation of criminal acts, as well as in the investigation of missing persons.

National DNA databases (primarily STR markers) hold more than 100 million DNA profiles distributed in some 60 countries around the world and are a key tool in national and international criminal investigations.

Spain regularly exchanges DNA profiles with 20 European countries. We are moving towards a global network of exchange of STR profiles in criminal investigation, as well as the development of new DNA databases with new markers, new technologies or new search strategies.

2nd Recommendation:

The growing international exchange of DNA profiles between countries with different legislations, as well as the expansion in the number of regions of DNA that may be analyzed in the future, suggests the development of standards of good practices in the management of forensic DNA databases to ensure: the right to protect genetic information and security and access measures in accordance with European data protection regulations, the right to cancel DNA profiles from innocent people, absolutions and similar situations (Marper Sentence of the European Court of Human Rights and recent sentence of the ECtHR against the French DNA database with fixed cancellation times of 40 years), the right to appeal would be safeguarded, the potential for "racial" or ethnic risk would be minimised, and a transparent management of DNA databases with publication of public memories would be carried out.

3- Likelihood Ratio:

A solid model for the interpretation of DNA evidence based on the use of Likelihood Ratio (LR) has also been built over the years, allowing **the integration, evaluation and comparison of DNA test results in each specific case** under the different hypotheses raised in the judicial process. The LR allows the interpretation of individual and complete DNA profiles as well as partial DNA profiles and DNA mixtures. It can also be applied in the field of criminal investigation, identification studies of corpses and missing persons, and genetic kinship studies.

The forensic geneticist offers the judge the value of the LR so that he/she can combine the information obtained in the DNA test with other non-genetic information obtained during the investigation. In doing so, we first take into account the perspectives of the accusation and the defence. The calculation of the LR therefore implies the establishment of two hypotheses regarding the facts, for example:

- Hp (accusation hypothesis) = the blood stain found at the crime scene belongs to the defendant.
- Hd (defence hypothesis) = the blood spot found at the crime scene does NOT belong to the defendant, but to another random person from the Spanish population.

The LR measures whether the DNA test results support one hypothesis more than the other. Suppose that the genetic profile found in the stain is complete and perfectly matches the profile found in the defendant's undoubted sample. In this case the LR value is usually very high (in the order of billions), which means that the DNA test strongly supports Hp.

But not all cases are like that. Sometimes other types of DNA are studied (mitochondrial, Y chromosome) that do not offer so much power of discrimination, or the genetic profiles obtained are of poor quality, or we detect very complex mixtures of DNA in which the uncertainty about who are the possible contributors to that mixture is high. In these cases, the LR values are not so overwhelming. Legal professionals must understand that **not all genetic test results are equal, and therefore they do not all equally support one hypothesis or another.**

On the other hand, in recent years, there has also been a great advance in the quantitative assessment of genetic profiles, mainly applied to mixtures and complex profiles that may present degradation, losses or gains allelic, and so on. At the same time, a lot of free software has been developed that allows statistical evaluation of this type of genetic results. Training and experience in this respect varies greatly from one expert to another, even within the same laboratory. It is therefore very important to constantly update the training of experts, since these advances make it possible to evaluate, for example, situations in which the genetic profile detected on the scene is not identical to that of the accused, but resembles that of the accused.

3rd Recommendation:

The use of likelihood ratios has become the standard for the evaluation of DNA evidence and is accepted by all laboratories and courts, as it allows a balanced (and neutral) interpretation of the probability of finding a genetic profile (complete or partial, individual or mixed) under the different hypotheses that may be raised in the judicial process (by the prosecution/accusation and the defence, fundamentally). Its use in the interpretation of other forensic evidence and analysis is also advisable.

4th Recommendation:

Understanding the concept of likelihood ratio and integrating the value of DNA testing with other tests is not intuitive and requires learning and training. Forensic experts must be trained in their correct calculation and communication and, on the other hand, legal professionals and especially judges and prosecutors, as well as police investigators, must be instructed in their correct understanding, in avoiding biases in interpretation (such as those known as the prosecutor's and defence's fallacy, or confusion between the error rate of the evidence and the LR) and in the correct integration of the value of the genetic test with other tests that cannot be so accurately quantified.

4- Background DNA and primary and secondary DNA transfers:

Today's PCR systems for the analysis of STR polymorphisms have increased their ability to detect ever smaller amounts of DNA. This means that tiny, invisible traces of DNA can now be retrieved and analysed without even being able to determine the cell type from which they originate.

On the other hand, in these years of application of forensic genetics we have learned that DNA can be transferred not only by body fluids, but also by the detachment of skin cells by contact with a surface or by creating aerosols when we talk, cough and sneeze, to name a few representative examples.

This **wide variety of DNA transfer mechanisms,** coupled with the **high sensitivity of current DNA analysis methods,** is one of the most complicating factors for both DNA analysis and the interpretation of DNA evidence in certain contexts, as there is a high probability that DNA recovered from a crime scene (in addition to the expected: that of the investigated) will be DNA from people who have had nothing to do with the crime. The DNA deposited before the crime and not related to it is known as "background DNA". The reality is even more complex as at the crime scene we may find DNA from someone who has never been there (DNA from a secondary transfer), secondarily transferred by another person who had contact (e.g. handshake) with the donor prior to visiting and depositing their DNA at the crime scene (e.g. by opening a doorknob with the hand with which they had previously touched the donor with). The possibility of transferring biological fluids between garments that are washed together in the washing machine, for example, is today a scientifically proven fact that warns us of the possibility that in our clothing we not only have DNA of ourselves, but also of the people who live with us.

Background DNA and DNA transfer mechanisms condition and complicate at least three aspects of expertise: (1) the need to discern between the DNA relevant to the case, and the DNA that is not, by means of discard studies with reference samples from potential contributors to the "background DNA" of the crime scene; (2) the additional technical and interpretative complexity involved in the analysis of mixtures of DNA profiles, in particular complex mixtures (more than two contributors) and (3) the difficulty of interpreting the meaning of DNA evidence in the context of a criminal investigation when the persons under investigation have had prior access to the crime scene and the presence of their DNA profile at the scene can be explained by an "innocent" transfer.

5th Recommendation:

The DNA test tells us with a high probability the individual origin of the evidence, but generally tells us nothing about how or when the evidence arrived at the scene of the crime. The possibility of detecting at the scene of the crime DNA profiles of persons who had nothing to do with the crime ("background DNA"), should be taken into account in the evaluation of the evidence especially in those DNA profiles obtained from biological evidence that can be easily transferred (by contact, aerosols...) or whose cellular nature could not be established in the forensic analysis. In other words, DNA identification in these cases does not have to be a guilty sign. The assessment of DNA evidence should therefore be made in the context of the case and in relation to all other forensic evidence, if any. Likewise, when the persons under investigation have had prior access to the crime scene, the possibility that the presence of their DNA profile at the crime scene could be explained by an "innocent" transfer should be assessed.

5- DNA contamination:

Another important element in the assessment of DNA evidence is the possibility of DNA contamination during the process of collecting biological evidence at the crime scene or during laboratory-analysis. The possibility of contamination arises from the very characteristics of the PCR technique used in DNA profiling, especially as a result of its high sensitivity to detect DNA profiles from only a few cells.

Despite the large number of measures implemented by the laboratories to minimize the possibility of contamination (separation of areas, use of material and reagents specific to each area, use of appropriate clothing and individual protection systems, work in biological safety booths, ...) today we know that it is virtually impossible to eradicate contamination completely, which makes it much more important to develop **measures to monitor and trace possible contaminations** that occur in the laboratory. Among these measures are the development of elimination databases of laboratory personnel, as well as professionals (police, forensic doctors, ...), who work in the collection of samples in order to make systematic comparisons of the profiles obtained in the real case with these databases to trace the origin of the contaminations. This situation also forces us to carry out a periodic review (at least annually) of the DNA contamination events detected by the laboratory, which allows us to document the possible mechanisms and causes of the contamination, as well as to identify the possible corrective measures to be taken and also to know the rate and types of annual contamination of each laboratory, which will give us a more objective idea of when contamination in a laboratory may compromise or limit the interpretation of the DNA test.

6th Recommendation:

Measures to monitor DNA contamination in forensic genetics laboratories become particularly important as it becomes virtually impossible to reduce the probability of DNA contamination in a forensic laboratory to zero. These measures include the development of elimination databases that are as extensive as possible, i.e. that not only contain the profiles of the DNA experts of the laboratories, but also the DNA profiles of all those professionals involved in the taking of samples and in any step of the investigation. Laboratories must also be aware of this possibility and, if it occurs, find out its causes and apply the appropriate corrective measures. Laboratories should also try to establish their annual DNA contamination rate.

6- Implementation of new massively parallel sequencing techniques and new DNA markers:

We are witnessing a new technological revolution in the field of forensic genetics. It is about the implementation in forensic genetics laboratories of the massively parallel sequencing methodology. Currently, there are a growing number of forensic genetics institutes and agencies that are investigating and beginning to implement within their laboratories this new mass sequencing technology for: (1) the analysis of "classical" forensic DNA markers (i.e., short tandem DNA repeats (STR) and mitochondrial DNA Control Region) used worldwide in forensic

casework; as well as (2) to study the possible application of other DNA markers (Single Nucleotide Polymorphism (SNP), which can be used for forensic individual identification studies, as well as in ancestry or biogeographic ancestry studies, as well as to determine some phenotypic characteristics (skin colour, eyes and hair colour) or even methylation studies to determine the biological age of the donor of the biological evidence that appeared at the scene of the crime. These new DNA markers (many of them located in genes or coding regions of the genome) open the door to new applications such as estimating the ancestry and phenotype of an individual prior to other DNA analysis in criminal investigation, or the development of new databases of biometric markers obtained from DNA in the identification of missing persons.

On the other hand, we are still far from being able to obtain a robot portrait of the killer from the biological trace found at the crime scene. And this is due, among other reasons, to the lack of current knowledge on the part of science about the genetic basis of the very complex human facial structure.

7th Recommendation:

The application of the new massively parallel sequencing technology and the new DNA markers, many of them located in genes that provide biometric information about our appearance will require a reform in the legal system of many European countries including Spain, whose regulations are expressly based on the study of non-coding repetitive DNA regions (STRs).

7- Forensic science research:

In contrast to other areas such as healthcare, (Health Research Fund), **the administration of justice does not traditionally invest in R&D&i.** All justice areas are affected by this concern, since no research is carried out to improve procedures and doctrinal work is not allowed to be oriented to the needs of real life. In the forensic area, the lack of investment has completely dramatic effects since the excellence of experts also requires an important investigation component.

In health area, improvements in life expectancy and quality of life, or in the quality of procedures and efficiency of health system has been largely a consequence of a systematic and sustained investment in research, innovation and development.

8th Recommendation:

It is recommended to provide forensic medicine, and forensic genetics in particular, with technical, human and economic resources needed to carry out research projects related to the Justice Administration.

3- Members of the working group:

The area of Science and Law is directed by: Antonio Garrigues Walker, Garrigues Foundation President, **Cristina Jiménez Savurido,** Fide´s President and **Pedro García Barreno,** PhD in Medicine and Professor Emeritus of the Complutense University.

This working group has been led by: Ángel Carracedo, Professor of Legal Medicine, Institute of Forensic Sciences, University of Santiago de Compostela; **Antonio Alonso Alonso,** Faculty member of the National Institute of Toxicology and Forensic Sciences and Member and Secretary of the National Commission for the forensic use of DNA and **Lourdes Prieto,** Collaborating Researcher, Institute of Forensic Sciences, University of Santiago de Compostela.

The members of this working group who have participated in this work of reflection and collective debate are: José Andradas Herranz, Facultative Officer of the National Police Force, DNA Database Administrator; **Jesús Agudo Ordoñez,** Director of the National Institute of Toxicology and Forensic Sciences, Department of Madrid**; Amaya Arnaiz Serrano,** Senior Lecturer, Universidad Carlos III de Madrid. Researcher at the Alonso Martínez Institute of Justice and Litigation; **Gemma Barroso Villareal,** National Police Commissioner, Head of the Central Unit for Scientific Analysis of the Scientific Police Station; **María Jesús Buitrago de Benito,** Forensic Specialist, Advisor to the General Bureau of Relations with the Administration of Justice; **Rosario Cospedal García,** Managing Director, GENOMICA S.A.U., Pharma Mar Group; **Jesús de la Morena Olías,** Managing Director, Garrigues Foundation; **Antonio del Moral García**, Magistrate, Supreme Court; **Juan Manuel Fernández Martinez,** Vocal of the General Council of the Judiciary; **José Miguel García Sagredo,** Full Academic Member, Royal National Academy of Medicine. Honorary Professor, University of Alcalá de Henares; **Amaya Gorostiza Langa,** Manager, Genetic Identification Laboratory GENOMICA S.A.U., Pharma Mar Group; **Eusebio López Reyes,** National Police Inspector, responsible for the DNA Database of the General Scientific Police Station; **José Juan Lucena Molina,** Civil Guard Colonel. Director, Civil Guard Specialisation School; **Víctor Moreno Catena,** Director of IAMJL. Professor of Procedural Law, Universidad Carlos III de Madrid. Lawyer. President, UEAP; **Mª Dolores Moreno Raymundo,** Forensic Doctor advisor to the Ministry of Justice; **Jaime Moreno Verdejo,** Public Prosecutor, Supreme Court; **Luis Rodríguez Ramos,** Professor of Criminal Law and Lawyer and **Ágata María Sanz Hermida,** Professor of Procedural Law, University of Castilla la Mancha.

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All the people who have participated in this working group of Fide, have done so in a personal capacity and not on behalf of institutions, professional offices, universities, or companies where they carry out their professional work, so **these conclusions do not reflect institutional positions but particular positions of each of the members of the group.**

4- Acknowledgements:

Fundación Fide and **Fundación Garrigues would like to thank all the members of the group** for having actively participated in the working sessions, introducing the topics for debate, contributing their experience, knowledge of the subject and their personal reflections.

We also thank: **Ángel Carracedo,** Professor of Legal Medicine, Institute of Forensic Sciences, University of Santiago de Compostela; **Antonio Alonso Alonso,** Faculty member of the National Institute of Toxicology and Forensic Sciences and Member and Secretary of the National Commission for the forensic use of DNA and **Lourdes Prieto,** Collaborating Researcher, Institute of Forensic Sciences, University of Santiago de Compostela, for the preparation of the recommendations document and the incorporation of the contributions of each of the members of the working group. We have attended debates of maximum interest and current importance and it has been an honour and a privilege to be able to count on everyone's contributions.

5- Science and Law Commission:

For almost five years now, **Fundación Fide and Fundación Garrigues** have been holding a series of **sessions** periodically, **bringing together scientists and jurists** to deal with a wide range of issues with the aim of **promoting lines of action and cooperation between scientists and jurists that are fruitful and, where appropriate, also constitute a source of legislative, training or other proposals useful for society as a whole.**

Both institutions have created the **Science and Law Commission**, whose aim is to advance research and knowledge in the fields of science which, due to their greater progress or complexity, require this dialogue. This Commission is directed by **Antonio Garrigues Walker,** Garrigues Foundation President, **Cristina Jiménez Savurido,** Fide´s President and **Pedro García Barreno,** PhD in Medicine and Professor Emeritus of the Complutense University.