Innovations in Forensic Genetics

Gábor Bihari[1], Attila Heinrich[2], Eszter Lőrincz [3]

The laboratories of the Department of Genetics use examinations to determine the human tissue/cell type origin, DNA profile as well as process bone and tooth samples from crime scenes and buccal swabs from individuals. The Department is currently involved in implementation of new methods to assist the investigating authorities in their investigations and further strengthening the admissibility of expert reports. The aim of these efforts is to handle degraded and latent small amount of DNA samples, successfully detect and statistically infer the minor component in complex "mixed" samples from multiple sources, and predict the eye colour, hair colour, skin colour and biogeographic origin of unknown individuals from crime scene samples. In recent years the increasing number of high-profile cases and the growing demand for examinations have posed challenges. The challenges have been addressed through code-based tracking and the implementation of a highly efficient and fast-processing automated multifunctional robot park. Overall, our goal is to increase examination capacity and reduce processing time in a cost-effective manner, thus optimizing the operation of the systems, concerning the ever-increasing number of cases.

Keywords: forensic genetics, multi-person mixtures, new methods

I. Introduction

The Hungarian Institute for Forensic Sciences (hereinafter referred to as the Institute) was established in 2017 through the merger of the Network of Forensic Sciences Institutes and the former Hungarian Institute for Forensic Sciences. The Department of Genetics including its predecessors has been conducting forensic genetic expert work since 1992. Our investigations aim to determine the origin of human tissue/cell types and human DNA-profiles from the samples received or specimens sampled by us. The majority of inquiries received by the Department come from police departments, with a smaller portion originating from prosecutor's offices and courts. Therefore, our clients and readers of our expert opinions are predominantly legal professionals and/or law enforcement officers. In this study, we attempt to provide an overview of the challenges of our departmental activities primarily intended for readers in the field of criminaltechnics.

[1] Gábor Bihari, forensic geneticist, Hungarian Institute for Forensic Sciences, Department of Genetics. E-mail: biharig@nszkk.gov.hu

[2] Attila Heinrich, forensic geneticist, head of Nuclear DNA Analysis Laboratory, Hungarian Institute for Forensic Sciences, Department of Genetics. E-mail: heinricha@nszkk.gov.hu, orcid.org/0009-0001-9229-1508

[3] Eszter Lőrincz, PhD student, University of Veterinary Medicine, Doctoral School of Veterinary Sciences, forensic geneticist, Hungarian Institute for Forensic Sciences, Department of Genetics. E-mail: lorincze@nszkk.gov.hu, orcid.org/0009-0001-0211-9027

II. Background

A What is accreditation and what is it for?

The backbone of our institutional activities consists of numerous work processes that are accredited, quality management permeates these systems, making it essential to describe its central role. The 22nd paragraph of the 12/2016 (V. 4.) decree of the Ministry of Interior requires that our laboratory activities and related tasks have a broad accreditation horizon, which is certified by the National Accreditation Authority (NAH).[4] To comply the MSZ EN ISO/IEC 17025:2018 complex quality management standard our processes such as: curriculum management, compliance with proficiency tests, supplier evaluations, investigation of non-compliances, corrective actions are needed.

The standard sets out the necessary criteria, which the National Accreditation Authority verifies and certifies that our activities meet the requirements of the standard. Quality assurance is only one aspect of the standard.

Back then, preparing for and then obtaining accredited status was a complex process. Even in an unchanged world, maintaining the status permanently would require continuous adjustment and renewal of the framework (for example assessment of suppliers, quality enhancement, integration of new experiences).

The framework has a preservation and integrative function, for example it can preserve and integrate individual experiences for example „masterstrokes" of expert competence and the otherwise relatively rare lessons of nonconformities (for example breakdowns, failures) that might otherwise sink into the drawer and be forgotten, thus building into a system the accumulated experience and knowledge from our large and changing pool of case experts, our significant case and sample numbers.

We believe that while continuously maintaining accredited status is no guarantee of best practice or infallibility, it inevitably creates and enhances a systemic internal commitment to quality, one that is organically embedded in the organisation.

The preservation of quality-managed status (as a constraint) by conspiratorial simulation of system outputs, by mimicking externalities - would require resource-intensive, irrational and risky duplications, "double-think" operations. We do not cover up errors and shortcomings that do occur, but seek to detect them and take measures to reduce or eliminate their recurrence. This is the so-called "*nonconformity management*", where the root cause is identified, the defect is corrected and corrective action is taken to prevent recurrence.

B How can we syncronise our workflow elements with the attached documents and generated data?

The majority of the data generated through our processes is integrated into our independently developed and maintained Laboratory Information Management System software (referred as LIMS). Our LIMS became a more and more extended system, improving the effectiveness.

Our experts can assign new examinations through the network based on the stored data and can generate and complete even the most complex expert reports. If quality management is the spirit or the backbone in the organization then LIMS is the nervous system.

[4] 12/2016, (V. 4.) BM rendelet para. 22

III. Overview

A The Process and Theoretical Structure of Our Material Examinations

Material examinations begin during the crime scene investigation when we can search for and presume the presence of biological material residues and secretions (for example, blood, semen) using chemical-based preliminary tests and forensic light sources (absorption, fluorescence), based on the characteristics of the crime scene or case documents. Not forgotten that unfortunately, some "*heuristics*"-"*cognitive bias*" will always be a part of this task. Further laboratory tests are then prescribed based on the received or recorded crime scene samples and the case documents.

The ultimate goal of laboratory examinations is the determination of the DNA profile, usually involving four steps.

(0) An optional zeroth preliminary step, which confirms the presence of human blood, saliva, or semen in the available sample material through the detection of tissue- and human-specific proteins.

(1) The first step involves the purification and concentration of DNA.

(2) The second step is the determination of the human DNA concentration in the purified sample.

(3) The third step involves the large-scale amplification of the specific features that make up the DNA profile using a biochemical chain reaction.

(4) The fourth step is the determination of the amplified features.

The basis for the second and third steps is the "*polymerase chain reaction*", which earned half of the 1993 Nobel Prize in Chemistry.[5]

In case of the reference samples (from persons) the entering point is the 3rd step -so this procedure is called "*direct amplification*".

In conclusion our examination process is multi-stage, contains multiple entering and endpoints and contains multiple feedback loops. Results obtained from steps 2 and 4 may justify the repetition of steps 1, 2-4, potentially with modified parameters.

- As our samples progress through the examination process, they become more abstract and unique from an informational perspective (unique DNA concentration and profile) at the same time they become more uniform in their material and physical properties. The physical products are clear DNA extracts with the same volume and pH, regardless of the specimen.

B The Spatial Structure of Our Material Examinations

We have to carry out the steps before and after the amplification of features in a spatially separated manner.

(i) Our low-copy-number (and mitochondrial) DNA laboratories

We focus on processing „latent" samples that can be presumed to contain human nuclear DNA in small quantities based on their external characteristics and case documents, such as bone or tooth origin. The proportion of samples entering the laboratory has significantly increased over the past decade, with currently around 9,500 samples per year, accounting for roughly 60 per cent of the processed on-site samples.

For DNA purification from bone and tooth samples originating from unknown individuals, there are spatial and temporal separations. Currently, there are two liquid nitrogen-cooled „bone mills" available for sample processing. DNA isolation from powdered samples is carried out on a *semi-*

[5] https://www.nobelprize.org/prizes/chemistry/1993/summary/

automated robot using factory-made chemical kits, and a manual organic extraction method is also available if needed.

(ii) Our high-copy-number laboratory

We determine the tissue/cell type origin of approximately 1,400 on-site samples and attempt profile analysis on around 3,700 on-site and 2,100 reference samples each year. To meet the increasing demand for examinations, the department has taken several optimization and development steps (as we discuss below) and introduced new methods.

(iii) Use of high-capacity, automated systems

Due to the increased demand for examinations, the laboratories use high-capacity, automated systems for DNA purification and amplification reactions. They are currently replacing aging robots with discontinued product support and planning future expansions in this area.
The testing of new DNA purification and quantification robots became available for the Institute in recent months and has been successful. We have emphasized the need for the devices' settings to be compatible with our currently used accredited procedures (for example, sample tube volume, DNA quantification, and requirements for the chemicals used in amplification), allowing easy integration into our system.

(iv) Transition to half-volume testing

Over the past few years, both laboratories have gradually introduced the so-called half-volume testing. The use of commercial, manufacturer-validated DNA profiling kits is performed at half the recommended volume. The clear advantage of *half-volume testing* is the halving of DNA sample and chemical consumption, allowing for more examinations to be conducted on the same DNA samples and significant cost savings (approximately 50 per cent) in the price of our most expensive chemicals.
Validation of the method with various tests has confirmed the reliability and performance of our half-volume procedures under different conditions.[6] We have found that the kits meet the manufacturer's recommendations and are suitable for routine examinations as well.
Half-volume profiling is an optimal compromise.[7] It achieves higher information density per unit of sample volume, while full-volume profiling is still available in our system.

(v) Transition to "direct amplification"

In case of the reference samples the oral swabs' entering point to the laboratory workflow is the 3rd step, so with *direct amplification* there is no further need for purification and concentration of DNA (1st step) or determination of the human DNA concentration (2nd step). So direct amplification is cost effective and time saving for oral swabs.

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[6] Eszter Lőrincz, Judit Zelei and Attila Heinrich, 'Half-Volume Optimization in Typing of Low Template Samples with PowerPlex Fusion 6C PCR Amplification Kit', poster presentation, *XX. Genetikai Műhelyek Magyarországon*, in english: *XX. "Genetical Workshops in Hungary" conference*, Szeged, (2021).
[7] Almohammed E. and Hadi S., 'Internal validation of GlobalFiler™ kit using reduced reaction volume', *Forensic Science International: Genetics Supplement Series, 7*(1) (2019) 878–83.

IV. Objectives and solutions

The efforts are centered around challenges such as:

A Objective I

Improving the efficiency of DNA profiling from samples containing small amounts of human DNA.

Obstacles encountered: on the one hand, crime scenes that are difficult to search visually or with classical instrumental and chemical techniques often have little human biological material residues - thus human DNA. On the other hand, the initially ideal amount of DNA released into the environment or present in the corpse starts to decay, so the DNA chains and the stored information also becomes increasingly fragmented - thus increasing the proportion of DNA chains - code sequences that are damaged and useless for our investigations.

By the time they reach our laboratory refrigerator, DNA chains are exposed to a wide variety of environmental influences that can damage and alter their structure. Examples include high temperatures, ultraviolet radiation, mould growth, bacterial activity, formalin soaking, the use of corrosive chemicals, boiling bones in bleach, various dactyloscopic reagents, superglue-cyanoacrylate fuming.

Improperly chosen handling and cleaning laboratory techniques can also damage and shorten DNA chains. Furthermore, long-term storage can also damage DNA.

The susceptibility of specificities to degradation varies due to their different DNA lengths so degradation events can hinder the amplification of the specificity to an exacerbating extent as the segment length increases.

Our Solutions:

(i) Lowering Quantitative Limits

Introduction of novel, more sensitive, and lower DNA input-demanding tests that can repeatedly determine DNA profiles from samples with limited quantities of human DNA.

(ii) Improving DNA Quality Limits

Improving DNA Quality Limits: Introduction of DNA profiling methods that require shorter intact DNA segments, such as next-generation sequencing or the use of two specific, complementary six-colour channel profiling tests (NGM Detect - GlobalFiler).[8]

(iii) Development of a Degradation Model

We have developed a *degradation model* to test and find the best testing methods for degraded samples. This model involves creating various sample standards of known degradation levels enzymatically and through ultrasonic fragmentation, starting from well-preserved, known-person DNA samples.[9]

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[8] Tamás Cseppentő, Katalin Rádóczy, Judit Zelei, Norbert Valis, István Aladzsity and Attila Heinrich, Optimization of two Forensic Multiplex PCR Kits in the Typing of Low Template & Degraded Samples, poster presentation, *XX. Genetikai Műhelyek Magyarországon*, in english: *XX. "Genetical Workshops in Hungary" conference*, Szeged, (2021).

[9] Gábor Bihari, Székely Éva and Dr. Heinrich Attila, 'DNS Degradációs Modell Fejlesztése Bomlott Minták STR Alapú DNS-Profil Meghatározásához', in english: „Developing a Degradational Model for the Profiling of Degraded Samples", poster presentation, *XX. Genetikai Műhelyek Magyarországon*, in english: *XX. "Genetical Workshops in Hungary" conference*, Szeged, (2021).

(iv) Multi-Colour Markers

Certain characteristics in the DNA profile can now be marked with five to seven different dyes in new-generation chemical kits (plus one colour reserved for "*Internal Lane Standard*"). During determination, these characteristics are detected on five or seven colour channels of the instrument. A testing system with multiple dye markers enables the concurrent determination of multiple characteristics, with a higher proportion of these being shorter chain lengths that are more resistant to degradation.

Analogy to trains: Think of the obtained raw DNA profile results as a few trains, with the characteristics being sorted into carriages according to their DNA chain length in the system. The shorter ones are placed in the front. The exposure of each carriage to damage is proportionate to its distance from the locomotive. There will always be „rear carriages" in the trains. However, we can choose to transport the characteristics in different, colour-coded trains to avoid mixing. With this logistics approach, more of the valuable cargo typically remains intact, usually the shorter characteristics in the front carriages. Another advantage is that various characteristics, assumed to be the same size in the analysis, can be transported in an identifiable manner due to different colour markings.

(v) Our Next Generation Sequencing

More precisely, *Massively Parallel Sequencing* goes deeper in resolution than our traditional DNA profiling tests; it provides additional information by determining DNA at the code level and examining nine more genetic markers, thereby increasing the discriminatory power.[10] The advantage of this method is that the characteristics under examination can be associated with smaller DNA fragments, which allows the method to work more effectively on degraded samples. According to the previous "analogy to trains" in this case there are no multiple rail carriages, each carriage/characteristic has its own locomotive.

In mixed - multi person samples another benefit is that, due to the DNA-code-level and massively parallel (up to 1000 times) reading, the characteristics of the minor component can still be determined even under shifted mixing ratios (till 1:19 ratios).

However, there are drawbacks to this method. Firstly, it is time-consuming, taking two to three weeks to complete. Additionally, it requires a significant amount of sample material, and the examination is limited to a maximum of 64 samples at a time. The cost of acquiring the necessary knowledge and equipment is also high. The establishment of a population database, as required for statistical calculations, is currently in progress, with the goal of analysing a population of 500 individuals. This will help determine the prevalence and composition of the examined characteristics within the population and assist in the identification process and result interpretation.

B Objective II

Distinguish between conditions that cause the DNA profile to diminish.

Problem: A fading profile towards longer DNA stretches may be caused not only by damage to DNA chains but also by substances that inhibit the amplification reaction (for example haematin from blood, humic acid from soil, metal ions). So the symptom - a fading DNA profile is not clearly inferred from the underlying cause.

[9] Balázs Kocsis, Mátrai Norbert and Egyed Balázs, 'Forensic Implications of the Discrepancies Caused between NGS and CE Results by New Microvariant Allele at Penta E Microsatellite', *Genes (Basel), 14*(5), (2023), 1109.

And the cause is important because while inhibitors can be removed by purification of the sample or their input to the reaction can be reduced by reducing the sample input, there is no reliable way to upgrade degraded samples thus partial regeneration of degraded DNA chains in a „reconstructive” amplification reaction is not practical for us.[11]

Our solution: The last generation chemistry kits we use, such as our kit for measuring human DNA concentration and our kit for degraded DNA profiling, allow us to assess two quality status indicators for samples. They allow the detection and thus the differentiation of two things; the degree of DNA degradation in the sample and the degree of inhibition resulted by the presence of substances that inhibit the profiling.

C Objective III

Detect spermatozoa even in samples containing only in minimal amount.

Problem: The detection of spermatozoa is a cornerstone of our expertise in the processing and evaluation of semen samples, but they are difficult to effectively detach from various cloth fabrics and can be obscured by other cellular components and debris during microscopic evaluation.

Our solution: SPERM HY-LITER™, which is about to be routinely introduced, is a new method specifically designed for forensic DNA laboratories, based on antibody proteins that specifically bind to human sperm heads. These proteins are labelled with a dye that fluoresces at a different wavelength when illuminated by a light source. The excitating wavelength filtered out from the optical path so the sensitivity of the method is so high that a single shining spermatozoa head can be easily and quickly identified on an apparently darkened microscope slide.[12]

D Objective IV

„*In silico veritas*” In the case of profile-matching, to estimate the probative value as reliably and accurately as possible, to reduce cognitive bias and to communicate the result in a comprehensible way.

Problem 1: Although single source, full match can be easily managed with the simple formula of Bayesian hypothesis testing (knowing the population frequency of each genetic marker), however the phrase „*full match*” may give more weight than high numerical probability value to a layperson's good-faith teleological (purpose-built) interpretation.

Our solution: Use a ladder-like verbal scale to place/classify the statistical results, where the rungs of the ladder are visually related to the number of inhabitants (small/large town, Hungarian country, humanity).

Problem 2: A large part of the real-world profiling is incomplete due to low DNA concentration or decomposition and multiple origins. The detection intensity of the features can range from extremely low to high.

Reason: Even in profiles that can be considered as single-person originated, extra genetic markers can emerge (“*drop-in”*) due to the sensitivity of the analysis and the sample’s past. They can also randomly disappear (“*drop-out”*) due to expected markers being lost when the sample has a low DNA content or is in a degraded state. These effects which distort profile matching can be particularly pronounced a multi-person complex profile.

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[11] Edward M Golenberg, Ann Bickel and Paul Weihs, ’Effect of Highly Fragmented DNA on PCR’, *Nucleic Acids Research*, Volume 24, Issue 24, (1 December 1996), Pages 5026–33.

[12] Takamura A, Watanabe K and Akutsu T, ’Advanced forensic validation for human spermatozoa identification using SPERM HY-LITER™ Express with quantitative image analysis’, *Int J Legal Med, 131*(4), (2017), 933–39.

Analogy to HIFI amplifiers: Think of the profiling process as a high gain biochemical amplifier on high volume so the originally small signal inputs as whispering may disappear, in addition noise level-the buzzing is increasing.

Our solutions:

(i) Predefined detection tresholds

The detection tresholds for signals that can be accepted as genetic markers are defined by validated standard parameters.

(ii) Compensating the distorting effect of randomness

The „*drop-out*" and „*drop-in*" of genetic markers, in other words the distorting effect of randomness, is compensated by repeatedly determining the DNA profile and evaluating the results in aggregate.

(iii) Taking into account the distorting effects of randomness

To evaluate the expert/software-detected profile mixtures, a so-called „*probabilistic*" software statistical evaluation is used, which takes into account the chances of random „*drop-out*" and „*drop-in*" events.

The second and third generation statistical software used for our calculations take into account the possibility of the „*drop-out*" and „*drop-in*" of genetic markers, and these events have a significant lowering impact on the result of the calculation.

Second-generation statistical software only takes into account the type and frequency of the characteristics included in the calculation within the population so they referred as a „*qualitative model*" based software.

In addition, with the third generation of more advanced software measures intensity values of the detected characteristics are also included in the analysis so they referred as „*quantitative model*" based software.

The programs estimates the proportions of contributors (known and unknown persons) in the hypothesized crime sample based on the resulting intensity values of several features in the profile, and then compare the fit between the expected values from the model constructed with respect to the proportions and the measured values - the more inaccurate the observed fit, the lower the resulting probability value, and, beyond a certain level of discrepancy in the fit, it gives a warning or an indication that the modelling has failed.

(iv) Implementation and verification of a state-of-the-art quantitative software

We have deployed and verificated one of the state-of-the-art quantitative forensic genetic software packages (DNAxs, Netherlands Forensic Institute) available, which is under continuous development and support.[13;14] We have also verificated two additional free tools (EuroForMix, EuroForMix Rep). Based on our experience, the new software usually gives on the one hand orders of magnitude higher probability values for real-based profile matches by calculating intensity

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[13] Füredi Sándor 'Kvantitatív statisztikai modellű személyazonosító szoftverek verifikálása a magyarországi bűnügyi DNS-vizsgálatokhoz', in english: „Verification of some Human Identification Software with Quantitative Statistical Model for Hungarian DNA Tests", poster presentation, *XXII.* "*Genetikai Műhelyek Magyarországon", in english: XXII. „Genetical Workshops in Hungary" conference,* Szeged, (2023).

[14] Corina C G Benschop, Jerry Hoogenboom, Pauline Hovers, Martin Slagter, Dennis Kruise, Raymond Parag, Kristy Steensma, Klaas Slooten, Jord H A Nagel, Patrick Dieltjes, Vincent van Marion, Heidi van Paassen, Jeroen de Jong, Christophe Creeten, Titia Sijen and Alexander L J Kneppers, 'DNAxs/DNAStatistX: Development and validation of a software suite for the data management and probabilistic interpretation of DNA profiles', *Forensic Sci Int Genet, 42, (2019),* 81–89.

values, and on the other hand, it often provides a strong support in the exclusion from suspicion of close relatives of the real, often unknown, contributor in multiple person profiles, who therefore have a similar DNA profile.

(v) Improved infographic presentations

An added benefit that our software package makes it much easier and quicker to review complex cases and complex profiles with its improved infographic presentations of results.[15]

(vi) Increased computational demands

Significantly increased computational demands result in long runtimes, our computing infrastructure is in progress.

(vii) Regularly informing the relevant ministry about the emerging potential of the available methods

Massively searching of complex DNA profiles that cannot be broken down into components is not allowed under national legislation, but from a technical point of view such searching would be possible, for example, by implementing a DNA database searching module of the software (SmartRank).

Apart from the legal constraints, from a purely technical point of view, in principle, an invaluable amount of investigative intelligence information suitable for further processing could be extracted from the entire domestic criminal DNA profile dataset by searching complex profiles and listing statistically plausible relationships of descent. Therefore, the Hungarian Institute for Forensic Sciences regularly informs the relevant ministry of the potential of the available professional tools.

E Objective V

Follow-up of samples across the full range of tests.

Problem: human tolerance for monotonicity is finite, error is low probability but inevitable, the theoretical possibility of sample substitution cannot be completely excluded on the basis of the paper trail of samples.

Our solutions:

(i) Exercising the "four eyes principle"

Many of the manual errors resulting from human error can be avoided and filtered out by using the „four eyes principle" and retrievable visual and video documentation and by repeating and robotising processes.

(ii) Building automated control, barcode based checkpoints and sample tracking to the manual workflow

Human error can be prevented through automated controls, for example some of our equipment requires scheduled maintenance and bar-coded scanning of chemical pack expiry dates.

In our workflows, we use barcode printers to mark unmarked tubes for sample confirmation and have implemented a barcode reader system to track barcoded sample tubes.

We incorporate software-assisted, barcode-validated verification steps into our manual workflow, so that barcodes on the sample tubes have to scanned during the process.

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[15] Corina CG Benschop and Sijen T, 'LoCIM-tool: An expert's assistant for inferring the major contributor's alleles in mixed consensus DNA profiles', *Forensic Sci Int Genet, 11,* (2014), 154–65.

F Objective VI

Improve the substantive accuracy and clarity of expert opinions.
According to Kertész (2004) "The legal practitioner uses an expert because he needs special expertise to understand the issue and finally receives an expert opinion which cannot be interpreted without special expertise."[16]

An obstacle has arisen: Our tools and equipment are calibrated and validated, our working procedures are validated and accredited, but the expert opinion resulting from the results obtained has to be finalised by the experts - experts cannot be accredited. There could be serious differences in the interpretation of the results, especially in the wording.

Our solutions:
To prevent typing errors and inattentiveness in the opinion, we use two automations;

(i) We have created a general pro-forma opinion body

(ii) The specific characteristics of the case are incorporated to the proforma

These are: case identification numbers, assignor's name, automatically generated result tables containing a number of measured, validated values. Furthermore the system recognises certain ranges of values - relevant text templates are incorporated.

(iii) Proofreading the document

The effects of language bias can occur on both the expert's and the reader's sides, so we have our colleagues proofread the completed expert opinion. We incorporate the experience of our hearings and external feedback into the proofreading feedback and automatic templates.

(iv) Providing recommended formulations for specific cases

For specific cases that cannot be detected by automation, we also provide recommended formulations.

Challenge: There is a growing need for statistical evaluation at higher levels of interpretation.
In the five-tier hierarchy of forensic hypothesis formation, our current activities extend up to the second level, which means we investigate hypotheses and performing calculations from the 1st „*sub-sub source level*" (individual profile components in mixtures) up to the 2nd „*sub-source level*" (characteristics defining the complete DNA profile).
At the 3rd „*source level*" (DNA quantity, degradation degree, laboratory contamination potential, visual appearance of the crime sample, preliminary test results) we report on the ground of the results -no statistical assessment involved.
Despite the areas of primary interest for most clients bounded to the 4th „*activity level*" and the 5th „*offence level*" we are not evaluating hypotheses at these levels of activity.[17]
„*The mistaken idea that answering the 'who' question, based on DNA profiling, is equivalent to answering the "who did it" question is widespread."* – Sheila Wills (personal communication).[18]

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[16] Imre Kertész in Kriminalisztika, (ed. Bócz Endre), BM Kiadó, Budapest, vol. I, (2004), 225.
[17] Duncan Taylor, Kokshoorn B and Biedermann A 'Evaluation of forensic genetics findings given activity level propositions: A review', Forensic Sci Int Genet, (2018 Sep), 36, 34-49.
[18] Ibid.

Emerging Response:
We closely follow the relevant results from the international literature, participate in related seminars, but as of now, we have not conducted statistical likelihood assessments regarding the upper three levels. The reasons for this limitation are the conservative nature of our field, the complexity of activity level hypothesis systems, the relatively young concept, the parameters involved in the equations requiring case simulations, cataloged observations, literature collections, and the need for specialized expertise in conducting calculations, which require time and careful consideration. Another difficulty that clients should have to formulate their questions about activity level very precisely and it can not be answered during a hearing.
Our activities are limited to the first three levels (1, 2, 3), and the laboratory processes associated with them are all validated and yield numerical or positive-negative results. The involvement of expert interpretation at these levels provides relatively little room for misinterpretation when a well-prepared expert interprets the results.[19]

G Possibility

Criminal phenotyping: new generation sequencing can be used to investigate characteristics that are linked to the so-called „biogeographical" origin, as well as those that are likely to be associated with certain external characteristics of the person, such as eye colour, hair colour, skin colour.[20] These can be complemented by the study of methylation patterns on DNA chains that change as a person ages, allowing an approximate determination of age from certain body fluids. The European *VISAGE* (VISible Attributes Through GEnomics) *Consortium*[21] offers chemical packages and support for all three questions, and we have the equipment and software to perform the tests, so VISAGE technology could be implemented in a year or two with explicit legal regulation.

V. Summary

The backbone of our activity is the accredited process system, whose documentation, the laboratory procedures and results are also managed by our extended Laboratory Information Management Software. These systems represents great value in the organization. Methods have been developed and implemented to differentiate and adequately treat a large number of different types of samples (Sperm HyLiter^{TM}, Next Generation Sequencing, Degradation Model). Cost-effectiveness has been significantly improved by the introduction of the *half-volume amplification* and the *direct amplification* procedures. Sample processing steps are monitored and controlled by barcodes, and increasingly by high throughput automated biorobots. From the detected profile matches we assess the probative value of evidence by extended quantitative model based state-of-the-art statistical software. We strive to minimize linguistic distortion and cognitive bias in the assessment and the communication of the results. In addition to the methods we use routinely, we also carry out scientific work and strive to monitor developments in our increasingly diverse field of expertise to implement the relevant techniques at a later stage.

[19] Amy M., Jeanguenat, Bruce Budowle and Itiel E. Dror, 'Strengthening Forensic DNA Decision Making Through a Better Understanding of the Influence of Cognitive Bias', *Science & Justice*, *57, 6, (2017)*, 415-20.
[20] Gabrielle Samuel and Barbara Prainsack, 'Forensic DNA phenotyping in Europe: Views "on the Ground" from Those Who Have a Professional Stake in the Technology', *Critical Studies of Contemporary Biosciences* 38, no 2 (2019), 119-41.
[21] https://www.visage-h2020.eu/#about

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