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Review

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Innovations in Forensic Sciences for Human Identification by DNA in the French Gendarmerie during the Last 10 Years

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Abstract: The IRCGN (Institut de Recherche Criminelle de la Gendarmerie Nationale) is a forensic science institute built by the French Gendarmerie which has the ability to exploit crime scene evidence. Any piece of evidence, anywhere in the world, in any environment, can be examined by IRCGN teams deployed in just a few hours. During the past 10 years, experts specializing in genetics have developed innovative genetic engineering technologies for application in forensic sciences. In this review, we highlight the main innovations and the creation of new tools for human identification, which are fully suited to the French Gendarmerie's needs. Devices developed by the IRCGN are specific to the Gendarmerie's purposes.

Keywords: genetic profiling; forensic science; innovation; NGS; microbial community

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1. Introduction

1.1. Genetic Profiling in Forensics and DNA Database in France

At the level of the individual, DNA is an extremely stable molecule, which guarantees that the integrity of the information it contains is preserved during cell divisions [1]. One of the methods commonly used in criminal investigations is DNA identification. For more than 30 years, the use of PCR has revolutionized forensic science [2], allowing genetic fingerprints to be obtained from traces containing little DNA or from degraded DNA. The genetic variations sought concern microsatellites, designated by the acronym STR (short tandem repeats)—short sequences of two to six nucleotide pairs repeated up to several tens of times [1,3]. The STRs are amplified by multiplex PCR using specific primers, one of which is coupled to a fluorochrome. The amplicons are then separated and detected using capillary electrophoresis [4–6]. These STR markers are highly polymorphic. In France, the discriminating power of this method is based on the combined analysis of 26 markers, which are described in article A38 of the Code of Criminal Procedure [7,8]. This analysis allows the determination of the probability of the appearance of a genetic profile in a reference population [9] of the order of one in several billions of billions. Only these markers can be used to feed the profiles referenced in the National Automated Genetic Fingerprint File (FNAEG), a national database created in France in 1998, described by article 706-54 of the Code of Criminal Procedure [10].

The FNAEG, therefore, makes it possible to centralize the genetic profiles obtained from the biological traces taken from the scenes of offenses and those of the individuals implicated or convicted [11,12]. Other types of genetic profiles can be registered at the FNAEG for specific cases (reference or relatives of a missing person, discovery of an unidentified corpse) [2,13]. During a reconciliation proposed by the file, confirmation

by an expert requires a statistical analysis based on the calculation of the probability that two individuals taken at random from the same population have the same genotype [2,3]. If the identification method involving STR genotyping seems relatively simple [14], the validation of genetic profiles can be seriously complicated when analyzing complex biological samples [15].

1.2. Forensic Genetic Laboratories in the French Gendarmerie

The IRCGN (Institut de Recherche Criminelle de la Gendarmerie Nationale) is a forensic science institute that has been devised according to the French Gendarmerie organization environment, in which forensic examinations are carried out and applied or experimental research works are conducted. The Institute is a unique model for several reasons. First, the multidisciplinary areas of expertise are all concentrated in one place. Second, the Institute can send forensic expert gendarmes anywhere in the world in just a few hours. Third, the Institute can strengthen Gendarmerie's operational teams or be joined by other specialized gendarmes. Over the last 10 years, this institute has developed strong genetic expertise in forensic sciences. French genetic expertise is recognized through the innovation and innovative methods developed in the "Forensic Genetic Biology Department".

This department is composed of three analytical units that proceed to biological analyses under different aspects and one DNA samples conservation unit. The High-Throughput DNA Unit for Individuals Samples is in charge of analyzing buccal samples from people subject to criminal prosecutions, which includes DNA profiling for integration in the National DNA Database (FNAEG). This unit is also in charge of analyzing reference samples from missing persons or relatives and identifying cadavers using DNA. Next, all the DNA samples collected at crime scenes are sent to the High-Throughput DNA Unit for Casework Samples, which is in charge of these analyses. The results are compared to the FNAEG to enable identification in case the offender's DNA is present in the database. This unit also deals with samples that could not have been standardized and conducts deeper analyses adapted to the nature of the sample. Finally, the Forensic Biology Experts unit is able to analyze all the results provided by the High-Throughput DNA Units. The DNA Sample Conservation Central Service stores the DNA samples from which DNA profiles have been determined but with no match in the FNAEG with an individual. Tens of thousands of samples are conserved in a controlled environment for several decades in case further analysis is required.

The objective of this review is to present the recent technical developments in forensic genetics and examples of which these new technologies have made it possible to optimize the chances of identifying victims or suspects in the French Gendarmerie during the last decade.

2. Innovations Made in the French Gendarmerie

2.1. Setup of a New Protocol for Disaster Victim Identification and Isolated Cadavers

2.1.1. Statement

When possible, the identification of a deceased person is carried out based on their known phenotypic characteristics (a passport photo, the presence of tattoos, etc.). Nevertheless, when identifying deceased persons, the INTERPOL protocol recognizes only three primary elements of identification (i.e., means of identification sufficient to pronounce a formal identification): fingerprints, odontology, and DNA fingerprinting [16].

Fingerprints currently remain the fastest way to identify a deceased person due both to the relative ease of taking fingerprints from the corpse (provided the fingers are still present and usable) and the possibility for comparison with reference fingerprints (found in the Judiciary Database, administrative databases, etc.) [16,17]. Forensic odontology also allows rapid identification provided that there are elements of dental comparison available (dental file, dental X-ray provided to the investigation services by the treating dentist (s), etc.) even when only a single tooth has been found in the area where the corpse was discovered [18–20].

Finally, in cases of extreme decomposition or destruction of the body (shredding or only pieces of muscle or bones available), the use of DNA identification is essential [21,22]. The recovery of reference samples is potentially simpler (compared to fingerprints, for example) since everyday objects can be used for this purpose. These may be recovered from the last places frequented by the victim (for example, their home) or those found at the site in the event of a disaster (personal effects in an identified closed suitcase, for example) [23]. In the absence of a reference object, a sample of cells (of buccal or blood origin) from the relatives of the supposed deceased person can also be taken by the investigators during contact with the families [24].

To recover biological material from the deceased person, a muscle fragment or bone fragment with flesh is usually sent to the laboratory to determine the genetic profile. Nevertheless, the samples available for analysis may come in different forms due to the sampling conditions (complete body or highly degraded pieces of unidentifiable origin, etc.) [25]. The success of DNA identification is closely linked to the methods of collecting samples from victims, storing them, and transporting them prior to analysis in the laboratory [26] while guaranteeing the cold chain [27]. In addition, in the event of a mass disaster with multiple victims and/or body parts, preservation and transport over greater or lesser distances require compatible logistics as close as possible to the disaster site, with the essential maintenance of the cold chain to prevent the degradation of the samples taken. These conditions are not often found at disaster sites [28].

To deal with these challenging questions, thanks to the accumulated experience in the High-Throughput DNA Unit for Individuals Samples, we started with the following observations made at the end of 2013:

1. The FTA® kit used in France [29] for signaling via biological sampling of identified individuals is a standardized sampling system consisting of the deposit of buccal cells on a cellulose matrix after rubbing a synthetic foam inside the cheeks of the individual [30];
2. As a corpse or body part is an inert biological material containing the potentially abundant genetic material, the FTA® card could be used to recover biological samples, depending on the level of decomposition of the corpse or the body element [30];
3. Transmission of the practice is facilitated as all Gendarmerie units have sampling kits;
4. Laboratory analysis can be accelerated thanks to the properties of the FTA® card (saving of 2 to 4 h for extraction), and automation allows mass analysis of samples in the event of a multitude of victims or specimens [31];
5. For laboratory technicians, the sample is dehumanized (a simple piece of paper), greatly reducing the psychological trauma inflicted during the treatment of a multitude of samples or body elements (morphologically identifiable or not) [32].

2.1.2. Initial Applications and Basics for the Standardized Cadaver Sampling Protocol

The first tests using this system were carried out in January 2013 during the investigation of an air crash in a forest near Grenoble involving five passengers on board (the father, the mother, and their three children) [7]. For four pieces of muscle determined as belonging to four different passengers, depending on the collection area of the disaster site, three sampling methods were tested (Figure 1):

- A piece of muscle analyzed by the method of digestion and DNA extraction;
- A transfer of organic matter via direct affixing of the piece of muscle onto an FTA® card, which was then analyzed;
- Wiping the piece of muscle using the sampling 'lollipop' from the standardized 'individual' kit, previously moistened with sterile pyrogen-free water, then transferring the sample by applying the lollipop to an FTA card ® for analysis.

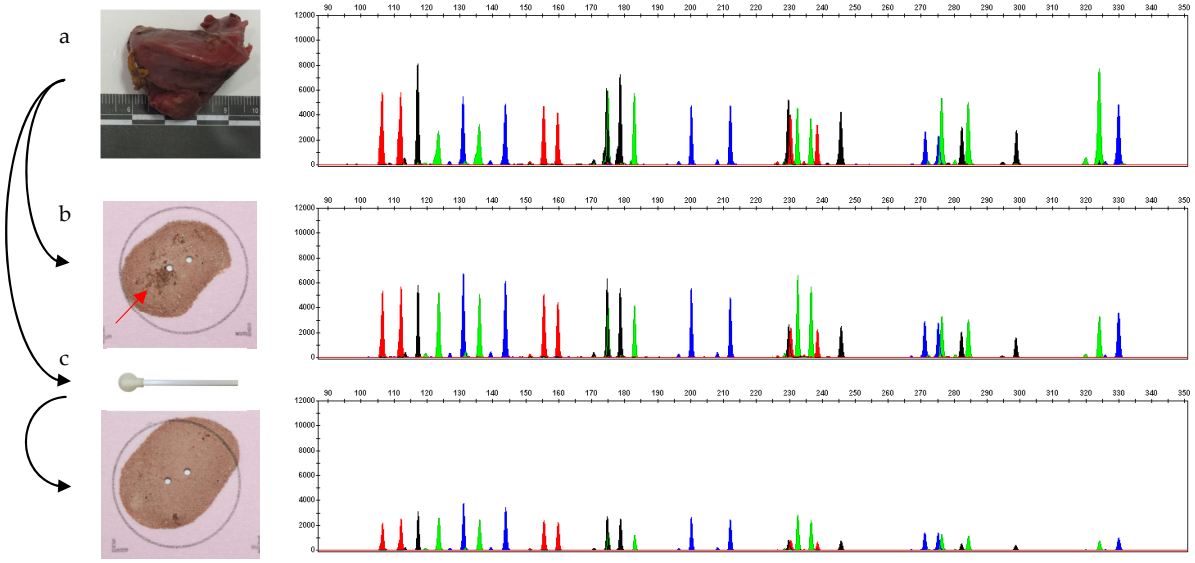

Figure 1. Sampling strategies and genetic profiles from: (**a**) analysis of a piece of muscle, (**b**) direct muscle application on FTA® paper (some muscle remains on the paper; see red arrow), analysis of a punch of 1.2 mm diameter, (**c**) muscle is wiped with a foam lollipop for application on FTA® paper, analysis of a punch of 1.2 mm diameter. Each analysis was performed in duplicate.

After PCR amplification of the DNA, sequencing on an ABI 3130 XL analyzer allowed a unique, complete genetic profile to be obtained for each sample, regardless of the DNA sampling and recovery method used [33]. Nevertheless, after affixing a piece of muscle directly to the FTA® paper, small muscle residues are present on the paper, hampering the use of the card in punching machines with a high risk of contamination of the punch head (shown by the red arrow in Figure 1b).

On August 2013, a single-engine plane crashed in a field not far from Vertaizon (63). The three crew members (two men and a woman) were killed, and their bodies were found dismembered. Due to the strength of the experimentation carried out on the former air disaster, 82 samples were taken using the standardized individual kit. The kit's sampling tool was used to wipe the pieces found and transfer them to an FTA® card.

All the identification operations involving genetic fingerprints were carried out within 48 h, including the reconciliation process [34].

2.1.3. Protocol Optimization

The use of the individual kit sampling tool was not optimal for reasons related to its foam head (Figure 2a). In order to optimize the sampling protocol before the transfer to FTA® paper, we tested the use of a swab whose head is covered with synthetic fibers flocked on the sampling tip, the 'Chemunex' swab from the Copan company, to bypass the faults of the foam (Figure 2b).

To make the most of the potential of this system and test it on a national scale, in August 2013, IRCGN provided Gendarmerie services with a standardized sampling protocol on corpses for identification purposes, which was based on genetic fingerprinting. From August 2013 to August 2014, 70 deceased persons (discovered dismembered, calcined, submerged, or putrefied) were identified using this protocol.

Subsequently, this protocol was implemented for the 'Air Algérie' disasters in the Mali desert (24 July 2014), Germanwings in the French Alps (24 March 2015), and a coach accident in the town of Puisseguin (23 October 2015) (Table 1), and was used for the identification of the victims of the attacks in Nice (24 July 2016, cf. §2.3). This protocol was presented during ISHI 2014, and similar techniques, which involved taking samples from cadavers using a swab for transfer onto FTA® paper for the purposes of genetic analysis, were subsequently published by the Parsons teams in the United States [35],

then by Montelius in Sweden [33]. The Hughes-Stamm team in the United States has also published work on the exploitation regarding achieving the transfer of biological material by directly affixing a piece of muscle on FTA® Elute paper [21,36].

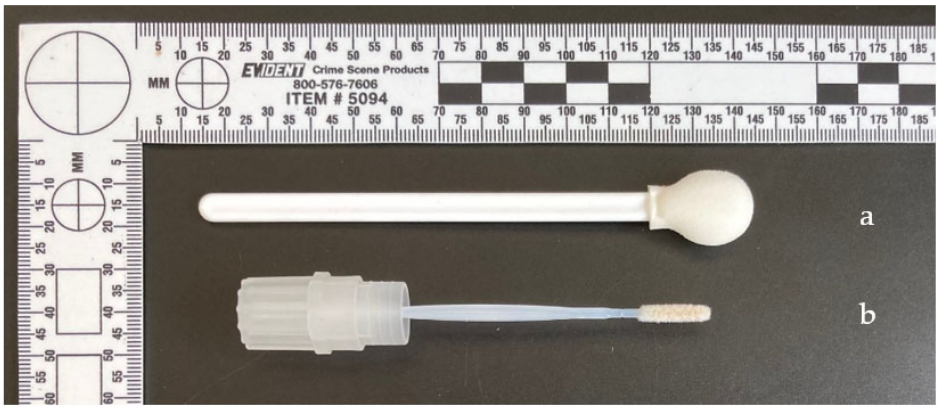

Figure 2. Main differences between (**a**) the foam lollipop of French FTA® kit and (**b**) a flocked swab used for biological trace collection. The foam head is larger than the flocked one and the swab is more adapted for recovering smaller liquid volumes and can be easily dried for further analysis if needed.

Table 1. Main disasters in 2014 and 2015, analyses performed, and genetic profile rates.

Type of Catastrophe	Number of Victims	FTA® Samples Analyzed	Success (%)	Swabs Analyzed	Success (%)
Air crash AH5017 (Mali, 2014)	115	40	93	130	76
Air crash 4U9525 (France, 2015)	150	2508	93	222	100
Road accident (France, 2015)	43	70	100	0	-

The corpse sampling protocol that we have developed is still used by the Gendarmerie units, as well as by the IRCGN during disaster victim identification missions, in synergy with the identification protocols using a genetic fingerprint from standardized bone samples or teeth. Indeed, we have also developed a patented device (FR2103443) called GenBones to perform bone sampling. It consists of a rapid, standardized, and easy-to-use system to collect and analyze bone samples. The collection method is performed using a trepan fixed on a rotary tool, such as an orthopedic surgery drill. The plugs of bone are length (up to 10 mm), diameter (2 mm), and weight standardized and are adapted for insertion in a microtube. Both protocols were used over several months on operational missions in Ukraine to identify victims of war crimes, allowing the Ukrainian population to benefit from the Gendarmerie's expertise.

2.2. Design of a New Collection Tool: The MicroFloq®

In order to optimize diagnostic work in forensics, IRCGN has patented (WO2016132028A1) an original DNA sampling tool, the microFLOQ® Direct swab (MFD), to make forensic DNA tests easier, faster, and allow them to be performed directly in the field. The microFLOQ® Direct Swab, first described in 2016 by the French Gendarmerie [37], was tested and validated by French experts and FBI experts [38]. Manufactured by the COPAN Company (Italy) and initially developed for forensic DNA analysis for the crime scene or disaster victim identification (DVI) context, the MFD swab is a miniaturized version of a floq swab that presents a 1 mm, two-swab head and a breaking point (see additional Figure 1b) that fit perfectly within a microtube or a 96-well PCR microplate. Flocked fibers at the head of the swab present a high affinity for nucleic acids and are embedded by lysing agents, allowing direct amplification and DNA analysis from a sample collection to a result in less than two hours. Additionally, the MFD swab subsamples only a small portion of the biological material (2 µL) and preserves the vast majority of the sample for subsequent

testing or reanalysis. These properties make the MFD swab a useful and smart device for rapid molecular screening. The microFLOQ® Direct Swab is able to collect small amounts of DNA from cotton cloth and may be considered as an alternate prescreening methodology in forensic biology casework [38].

2.3. Design of the First Mobile Lab for Genetic Analysis

Feedback from the mission to identify the victims of the Air Algérie disaster in Mali has led to internal reflection both on the organization of the analytical chain of post-mortem samples in the laboratory and on transport times. Indeed, the repatriation of the samples to French territory considerably increased the delays in identifying the victims, adding to the non-compressible delays for the analysis and interpretation of the laboratory.

Thus, during the mission to identify the victims of the Germanwings 4U9525 air disaster in March 2015, we experimented with the concept of a mobile genetic analysis laboratory by using the IRCGN laboratory bus [39] to transport all the material needed for sampling FTA® cards, as well as for amplification and DNA sequencing (Figure 3). This experiment was possible thanks to the greater robustness of the 3500 XL sequencer compared to the laboratory's previous model, the 3130 XL [39].

(**a**) (**b**)

Figure 3. (**a**) French Gendarmerie Forensic Laboratory bus (**b**) fitted for DNA analysis with dedicated areas for drying and sampling FTA® cards (foreground) and (**b**) for performing analyses (background).

The results obtained in the laboratory bus could not be used to identify the victims due to regulations in France concerning genetic analysis laboratories and the need for the approval of the Interministerial Approval Commission (cf. decree n° 97–109 of 6 February 1997) [39]. Nevertheless, around one hundred standardized samples were successfully analyzed directly at the site, demonstrating the feasibility of the concept, but with two constraints linked to the IRCGN laboratory bus:

- The versatility of the missions carried out in the vehicle. It is necessary to have a mobile structure dedicated to genetic analyses in order to limit contamination and, on the other hand, to design a specific environment for each analytical step [1] (before and after PCR amplification);
- The size of the vehicle makes it difficult to transport on narrow roads. The same is true for parking, which requires a location and sufficient surface area for deployment and installation [27]. Finally, it is a vehicle requiring a specific driving license, limiting the number of potential drivers.

In order to overcome these two constraints, we designed the first prototype of a mobile DNA analysis laboratory (LabDNA) based on a Renault Master L3H2 type. Patented innovation from the IRCGN [40], the first mobile DNA analysis laboratory (LabDNA), offers new potential in the field of identification by genetic fingerprinting (Figure 4a).

Figure 4. (**a**) The first mobile DNA Laboratory is made of a Renault Master L3H2 fitted for DNA analysis. It allows transport of all the equipment in the field. Once deployed, different areas are dedicated to an analytical step, limiting the risk of DNA contamination. (**b**) The mobile DNA laboratory version 2 is a container sealed on a van which is more compact and does not need side tents to be deployed.

Associated with another patented Gendarmerie innovation, the MFD swab [37] constitutes a unique technical setup capable of producing genetic analyses fast and at high speed as close as possible to the area of serious crime (multiplicity of bloody traces), an attack or a mass accident, and to integrate the results obtained into the judicial or extrajudicial procedure for identifying victims [34].

It was used for the first time in Nice as part of the mission to identify the victims of the attacks of 14 July 2016 [40]. This Mobil'DNA laboratory is a fully autonomous and adaptable ISO 17025-certified mobile laboratory used to perform genetic analyses in the context of crime scenes, terrorist attacks, or disasters.

Following this first prototype, version 2 of the Mobil'DNA was designed (Figure 4b) and used in 2020 to support the hospital task force in Paris during the peak of the COVID-19 pandemic. It was adapted to perform high-throughput molecular screening for coronavirus SARS-CoV-2 via real-time PCR to assist in Coronavirus SARS-CoV-2 diagnosis [39]. In 2022, this Mobil'DNA lab was used twice over several months on operational missions in Ukraine to identify victims of war crimes, allowing the Ukrainian population to benefit from the Gendarmerie's expertise. France also offered a Mobil'DNA laboratory to Ukraine in the same year to further the transfer of expertise.

2.4. Design of a New Swab Sampling Automaton

One of the missions of the Gendarmerie's forensic genetics laboratories is the high-throughput analysis of hundreds of DNA traces collected with swabs at crime scenes. Thus, hundreds of swabs are processed daily. One main challenge is to ensure traceability and avoid cross-contamination during the step of sampling swabs in a 96-well plate. Based on our experience, we designed and patented a new system called SPS (Sample Positioning System) (Figure 5a) (patent ATEPS FR2000959; SPS FR200516).

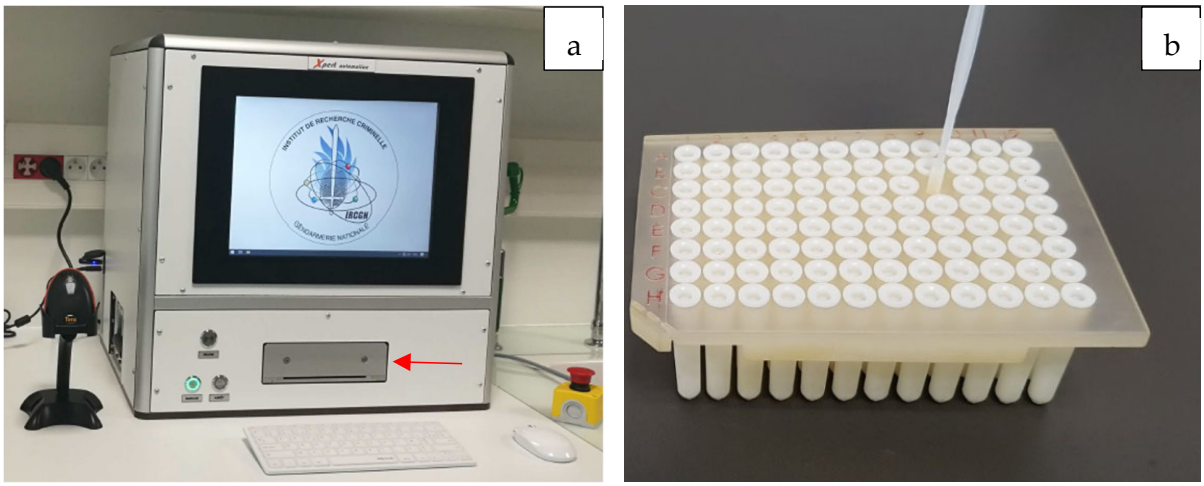

Figure 5. (**a**) The SPS (Sample Positioning System) automaton (Xpert automation) is dedicated to the sampling of swabs in specially designed 96-well plates (**b**). This system allows the sampling of hundreds of swabs per day with high traceability and without cross-contamination from well to well.

The SPS automaton allows the sampling of swabs in 96-well plates in compliance with ISO 17025 accreditation standards, ensuring the quality of the results. The whole plate is covered with plugs that automatically draw out one well at a time, depositing the sample in the right place and detecting its absence if necessary (Figure 5b). For this purpose, the automated machine has a harpoon that removes the hook from the well of interest, according to a plate plan initially chosen, and then presents the plate to the operator by opening the drawer (see the red arrows in Figure 5a). The operator will then place the sample in the only open well without risk of contamination, as all other wells in the plate remain closed. After validation of sampling on the touch screen, the drawer closes. The automated verification of the actual presence of a sample is carried out by an integrated camera inside the device. If the sample is present, the machine opens the next well and continues the process. If not, the automaton presents the plate to the technician for verification and to resolve the issue. A computer connected to the automaton ensures the traceability of the processing of the plate.

3. New Innovative Technologies Adapted for Forensic Sciences

3.1. The Revolution of Microfluidic Technologies: The Rapid HIT™ Technology

Next, we will discuss the analytical capability of RapidHIT™ 200 [41,42] for four kinds of routine forensic samples and the recyclability of templates, template DNA, and PCR products in the process of twice duplicate detection. In this machine technology, the STR loci could be detected in the buccal swabs by the system for the first time [43]. The RapidHIT™ ID system produces GlobalFiler™ analysis results after a short operating time. This device is effective because it automatically extracts DNA from buccal cells or blood stains and saliva collected at a crime scene [42], with a subsequent polymerase chain reaction performed to produce a DNA profile. Two types of dedicated cartridges are available for RapidHIT™ ID: the RapidHIT™ ID ACE GlobalFiler Express sample cartridge for references and the RapidINTEL™ sample cartridge for biological traces, such as blood stains [44]. Previously validated specimens include oral mucosa cells and bloodstains left at crime scenes. There have been no reports of blood and nail clipping samples collected from the postmortem bodies at the time of death. This report summarizes the results of using the RapidHIT™ ID system by collecting a variety of actual forensic samples from postmortem bodies at different stages of decomposition, which were subsequently analyzed using these cartridges [45].

Rapid DNA instruments are gaining interest in the forensic community as a means of generating DNA profile information more quickly than standard laboratory workflows, and

with the potential to be carried out at the scene from which samples are taken. In general, profiles produced using the RapidHIT ID system showed good discrimination power [45] but less than those produced via the standard laboratory procedures [41].

This system is currently used overseas (New Caledonia, Mayotte, Guadeloupe, and French Guyana) and has demonstrable effectiveness in the context of investigations under flagrante delicto. Analysis of the trace swab is carried out on the automaton [42,46]; the results are transmitted by an encrypted/encrypted telematics link to an IRCGN expert in France for the interpretation and rendering of the result in order to comply with the regulations. The use of this latest-generation technology in genetic analysis will revolutionize the work of experts in the pursuit of justice. Transversal validations are being studied to allow the deployment of this system on a large scale.

3.2. NGS Applied for Forensic Analysis

Over the last few decades, advances in sequencing have improved greatly [47]. One of the most important achievements of Next-Generation Sequencing (NGS) is to produce millions of sequence reads in a short period of time [48] and to produce large sequences of DNA in fragments of any size [49]. Libraries can be generated from whole genomes or any DNA or RNA region of interest without the need to know its sequence beforehand [50]. This allows the search for variations and facilitates genetic identification [51].

NGS technologies and their application would be especially useful in the forensics field. One of the main problems associated with forensics is the limited amount of samples available, as well as their degraded state. With the use of NGS, it will be possible to achieve simultaneous analysis of the standard autosomal DNA (STR and SNP) [52], mitochondrial DNA, and X and Y chromosomal markers. The top challenges of adopting new methods of forensic DNA analysis in routine laboratories are the capital investment and the expertise required to implement and validate such methods locally [53]. In the case of next-generation sequencing, in the last decade, several specifically forensic commercial options have become available [54], offering reliable and validated solutions. If the amount of DNA input required for preparing NGS libraries continues to decrease, nearly any sample could be sequenced; therefore, the maximum information from any biological remains could be obtained [55].

Besides their benefits in DNA profiling, NGS technologies allowed the development of new methods leading to the production of DNA intelligence through SNP analysis (i.e., the ability to acquire intelligence on a person through DNA analysis). In this way, forensic DNA phenotyping (FDP) tended to be more and more democratized during the last decade. This practice was authorized in France in 2014. FDP consists of the prediction of externally visible characteristics (ECV) of an individual (e.g., eye, hair, skin pigmentation). An example of the use of such information is the refinement of anthropological facial reconstruction [56]. SNP analysis can also allow an inference of the geographic ancestry of an individual. Taken together, this information can guide investigators to new leads or narrow the field of investigations, as a witness testimony does. Continuous technology developments and legal evolution should allow the precision and the addition of predicted characteristics in the next few years.

The recent progress in epigenomics provides another opportunity to collect information from DNA. Indeed, the most promising method of estimating the age of an individual is the analysis of DNA methylation when analyzing biological samples [49,57,58]. This prediction method can be crucial when only DNA traces are left at the crime scene or in order to complete an age estimation from human remains. Age prediction based on DNA methylation relies on age-related hypomethylation or hypermethylation of particular cytosines followed by a guanine (CpG site) found in CpG islands (i.e., DNA sequence containing > 55% CpGs) [59]. In recent years, many forensic-focused age prediction models were developed based on DNA methylation analysis using different analytical platforms, statistical analysis methods, and tissue types [57]. The main approach for the CpG sites methylation rate determination consists of targeted bisulfite-converted DNA sequencing.

This corresponds to the conversion of unmethylated cytosines into uracils, coupled with targeted amplification and DNA sequencing. If pyrosequencing and single-base extension can be considered the gold standard for sequence analysis [57,60], new developments in NGS could enable an increase in sensitivity and precision in predictions [61,62]. In fact, being a quantitative method, DNA methylation analysis presents the disadvantage of demanding a large amount of DNA in order to obtain a reliable prediction. The multiplexing ability of NGS methods could help decrease the amount of DNA necessary for the analysis.

3.3. The Microbial Communities: New Perspectives in Forensic Sciences

The objective of forensic sciences is to find clues at a crime scene in order to reconstruct the scenario [63]. Classical samples include DNA or fingerprints, but both have inherent limitations and can be uninformative. Another type of sample has emerged recently in the form of the microbiome. Supported by the Human Microbiome Project, the characteristics of the microbial communities provide real potential in forensics [64]. They are highly specific and can be used to differentiate and classify the originating body site of a human biological trace. Skin microbiota is also highly specific and different between individuals, leading to its potential as an identification tool [65]. By extension, the possibility of microbial communities being deposited on everyday objects has also been explored. Other uses include the determination of the post-mortem interval or the analysis of soil communities [11]. One challenge is that the microbiome changes over time and can be influenced by many environmental and lifestyle factors. This review offers an overview of the main methods and applications to demonstrate the benefit of the microbiome to provide forensically relevant information [64]

4. Conclusions

In the last decade, significant innovations have been made in the French Gendarmerie to help investigators. While the technology of obtaining a genetic profile from a DNA trace is constantly increasing in sensitivity, one of our challenges is now to accelerate the interpretation of the results to provide information to investigators, in particular for the High-Throughput DNA Units. One of the ways of innovation can be the use of artificial intelligence to help or even replace the expert under certain conditions.

Another challenge is to improve the processing of touch DNA traces. Analyzing touch DNA samples represents several challenges. Unlike blood traces, they are difficult to detect. Consequently, these samples are often collected blindly. Generally, police officers and other specialists use a deductive approach and try to figure out what object the offender might have been in contact with in order to define the areas of interest to be collected. Consequently, it is possible to target a surface harboring only a few cells (or no cells at all) while swabbing and to neglect a neighboring surface where many cells are present. Yet, touch DNA traces represent the vast majority of samples analyzed worldwide within forensic genetic laboratories. For instance, 80% of the thousands of biological traces analyzed in the IRCGN are touch DNA samples. Despite the crucial role they may play in criminal investigations, no DNA profiles were obtained for 50% of these samples. Similar success rates were reported in other DNA laboratories. To improve the success rate of analyzing touch DNA traces, the collaborative Biotrack program was set up with partners from Switzerland (University Center of legal medicine—forensic genetic unit (Lausanne), Institute of forensic medicine (Zurich)) and France (IRCGN (Pontoise), CY Cergy Paris University—ERRMECe and ETIS (Cergy), Ecole de l'ADN (Nîmes), Clotho AI (Chambourcy)). It consists of a collaborative program combining a set of academic partners with knowledge in cell biology and biological interactions with biotic or abiotic surfaces, as well as genetic and forensic scientists. This program aims at gaining insight into the biology of touch DNA and improving the success rate of DNA profiling. It is currently led by the Gendarmerie.

Finally, recent uses of recreational databases to identify individuals in criminal cases in the United States open up a new exploration path for the use of NGS applied to forensic

genealogy. However, national regulations must adapt to these new opportunities to be in compliance with the conditions for the uptake of a profile in the DNA databank, the right-of-Contra investigation, and the retention period of reference DNA.

5. Patents

In this work, 14 patents were established:

- DISPOSITIF DE STOCKAGE À TEMPÉRATURE AMBIANTE DE MATÉRIELS BIOLOGIQUES FR2019000016 HUBAC, Sylvain
- DISPOSITIF DE COLLECTION DE MATÉRIEL BIOLOGIQUE À PARTIR D'UNE TRACE BIOLOGIQUE FR2016000026 HUBAC, Sylvain
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