Chapter 8

Most Common Medico-Legal Autopsy-Related Human and Nonhuman Biological Samples for DNA Analysis

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Additional information is available at the end of the chapter

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Abstract

The identification and individualization of biological evidences is crucial to actual criminal investigations. In spite of the differences at the national level, all the legal processes attribute particular importance to forensic DNA analysis. However, none of the qualified results from any professional laboratory can produce substantive, valuable evidence with insufficient quality of samples and/or problems with provision of a pristine and controlled environment. The methodology and efficiency of sampling are distinct in case of living persons and in medico-legal autopsy and crime scenes. This chapter is a short overview from the basic introductory information up to ongoing research, and in accordance with constraints on the chapter size, it briefly discusses the important topics of sample collection at medico-legal autopsy for DNA analysis. The content sorts the major types of samples, reviews the common methods of sampling and the potential risk of poor sampling or contamination transfer. The corpses can be more or less degraded, which in special cases (e.g., paraffin embedded tissues, drowned, burning and/or buried cadaver) allow only for analysis of highly degraded samples. The samples can be associated with tissues of a corpse (e.g., blood, soft tissues, bone, tooth, hair) and/or additional extraneous tissues and remains, which are often mixed (e.g., blood, saliva, semen, vaginal fluid, debris of fingernails) on the corpse.

Keywords: medico-legal autopsy, molecular autopsy, autopsy sampling, contamination transfer, forensic genetics

1. Introduction

Medico-legal autopsies usually provide information in connection with violent acts and may provide relevant insight into cases of suicidal, accidental, or unnatural death. The relative volume of autopsies performed can, however, vary from country to country depending on their particular legislation [1]. The classical methodology can nowadays be either partially replaced or may be complemented using modern imaging technologies [2]. The procedures involved in medico-legal autopsies may include the death-scene investigation as well as the ancillary examinations. The demand for better clarifications concerning manner of death have extended the field toward molecular—genetic autopsy [3].

The principal goal of postmortem crime scene examinations and subsequent medico-legal autopsies is to determine, as precisely as possible, the time, cause, and unique circumstances of death. In these proceedings, from the onset of the official crime scene investigation to the conclusion of the professional autopsy performed later in a morgue, close cooperation between the investigation authority and designated forensic medical experts can work to provide the most-accurate answers to these critical questions. The extent of this cooperation must begin by working together at the crime scene through the preservation and evaluation of information gathered during examination and autopsy of the corpse in the morgue. This cooperation is of most importance in cases of violent deaths, where the primary goal is the determination and apprehension of the putative offender as quickly as possible and eventually leading to a subsequent conviction based on irrefutable and verifiable biological evidence.

Connection of a perpetrator to a victim and/or demonstrability of association within the given crime scene, i.e., a proof that a specific act was, or could have been, perpetrated by that individual greatly depends on the amount and quality of physical evidence provided by the authorities. In other words, it depends on the degree of validity of the evidence collected. In most cases, one of the most informative elements of physical evidence is the volume of biological remains/samples that have been secured and preserved at the scene, or during the autopsy and made available for expert examination at a later time. Some of these biological samples may assist in positively associating the perpetrator with the crime scene, while others may confirm the direct participation of an individual in an act of violence which was committed.

These aspects collectively emphasize the absolute necessity of implementing the official procedures as closely as possible in order to effectively procure and preserve contamination-free samples of relevant biological remains. This necessity extends to autopsies as well. The importance of using proper sampling methods during medico-legal procedures cannot be overemphasized. Mitigating the risk of contamination is especially important due to the fact that it may influence expert evaluation and precise interpretation of the results of later laboratory tests. In connection with this, it has also been proven to be important to be able to defend a case against possible legal attacks on the verifiability of these proofs based solely on the method of their procurement, storage, or handling. In regards to contamination, results of an examination can exhibit not only those pertaining to the person or another living being, e.g., animal or plant life, from which the specimen had originally originated, but may also detect postmortem accumulated biological traces acquired through the movement of the corpse, contact during its examination with other people, animals, plants, or other contaminated surfaces, objects, or devices. This is especially relevant in cases of decomposed bodies [4, 5].

The possibility and relevancy of contamination must always be considered. The chance of its occurrence cannot be fully excluded even in the most careful and circumspect proceedings,

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although we must also note that an insufficiently careful process of decontamination of the body —even on the dissection table—may also result in the elimination of relevant microtraces. With the application of suitable regulations and protocols, the risk can, however, be minimized.

This practice can be important, especially in the cases where a resuscitation attempt was made prior to the crime scene investigation, or when, during the time between the crime scene investigation and the expert autopsy, other individuals may have intervened—for example, manipulation of the corpse during shipment, or there is contact with the pathologists themselves. An increased risk is also present in the case of such practice where the establishment of an isolated infrastructure for the autopsy of a violent death victim in an external location, e.g., graveyard, is either not possible or not entirely suitable, as well as in cases where, for example, in pathology departments or teaching facilities, dissections from multiple cases are performed one after another.

The medico-legal autopsy is one of the first steps in the identification of biological remains. The first, earliest step of characterization of biological evidence in every autopsy room is usually the visual observation with or without the usage of special light sources. For indication of putative nonvictim biological remains, presumptive tests can be applied, followed by an adequate confirmatory test. The proper application of these tests may often result in optimal sampling, however, in the autopsy room, the risk of secondary transfer during manipulation may be elevated [6–9]. The heterogeneity of potential biological evidences even under slightly varying conditions and quality assurance of medico-legal autopsies [10–14], in addition to scientific considerations and practical experience, may also demand a kind of predictive common sense for deliberation during the sampling process to find the optimal result in context of necessary and satisfactory requirements.

2. Most common samples of autopsy for DNA examination, considerations, and characteristics of DNA samplings from victims of violent death

2.1. Victims of sexual assaults (VSA)

In cases of sexual homicide [15–17], in contrast to other forms of homicide—e.g., carried out using a knife or firearm—before the onset of violent death, the offender and the victim are usually in physical contact for a protracted period of time. During this time, even prior to the violent sexual act, the perpetrator attempts to use the true or perceived position of physical power to render the victim unable to act or escape and/or to place them in a state where they cannot defend themselves, as well as to keep them in that state while warding off any attempts at self-defense. In many cases, this is accomplished by covering the mouth and nasal openings —gagging—or grasping of the neck and choking. This series of actions may be accompanied in many cases by stuffing of the external airways and mouth with material. The perpetrator may kiss many parts of the victim’s body, may hit the victim, rip their hair as they grasp them, or violently rip off their clothes. The victim may be forcefully penetrated in his/her mouth, anus, or vagina [9], and then, the sexual act may occur involving anal or vaginal penetration—with or without ejaculation [18].

At every stage mentioned above, there is the possibility for the victim and perpetrator to leave biological samples on each other—epithelial cells, saliva, semen, hairs, blood, tears, sweat, urine, or feces. During the victim's attempt at self-protection, the perpetrator most often leaves biological samples of skin, head or body hair and/or saliva on the victim, or under his/her fingernails [19]. The victim may inflict a—possibly bleeding—wound on the perpetrator by biting some parts of his/her body, or biting off, and perhaps even swallowing some piece of a body part. In many cases, the violence is carried out with a partner or in a group, in which case, biological samples from multiple individuals may be found mixed on the victim's body or in some body orifice of the victim, or the victim's self-defense may have been directed at multiple persons. Whatever the case, even in the case of a single attacker, it is unlikely for samples obtainable from the body surface to be of homogeneous origin, while in the case of samples from body orifices, it is almost unavoidable that the biological samples connected to the potential perpetrator be mixed [20]. The careful collection of samples contributes greatly toward increasing the chance for conclusivity of subsequent laboratory tests and/or expert proceedings [21]. Conversely, a lack of care taken in the collection of samples could certainly preclude these results.

Mixed samples found on the victim's clothes, body, or surroundings—animal hairs, plant compounds, earth particles, microorganisms, fungi, etc.—are also preserved from the surroundings where the body was located between the time of the assault and its discovery. The later examination of these can provide important incriminating proof for the criminal procedure [22]. The relevant samples from the perpetrator are usually found in a more homogeneous form on the clothes of the victim than on the body. The corpse may additionally have been washed, denuded, redressed or wrapped, or dragged/transported to a location remote from the site of the crime, or even dismembered.

One of the possible motives for sexual homicide is unfaithfulness, or the suspicion thereof. Consequently, a compulsory part of the autopsy is the examination of the uterus, or in the case where pregnancy is detected, the securing of a biological sample linked to the fetus. This sample may be—depending on the age of the fetus and the postmortem interval—from uterine wall scrapings, placenta, the fetus, or any tissue, organ, blood, umbilical cord, bone, or any other fetal internal organs which contain the DNA of the terminated fetus [23]. In the case of uterine wall scrapings, avoiding the mixture of the mother-fetus samples requires special care, especially in the case of family—genetically related—violence since the mixed genetic profile can influence the statistical interpretation of putative fatherhood [24, 25].

On the basis of the above information, procurement of samples from multiple body surfaces is important—for example, samples from hair, external sexual organs, anus, mouth and nasal orifices, or from the ear region, or from the areas of gripping injury marks on the neck, thigh, or upper arm or internal surfaces may be justified and the relevance of foreign substance remains detected on the skin or fingernails or scratch marks is obvious. Samples may be taken from body cavities—from vaginal and anal cavities, the oral cavity and in some cases from the stomach. Foreign materials used to close off airways or placed in the oral cavity may also carry biological material associated with the perpetrator and, in case of a pregnant victim, the gathering of fetal samples—uterine scrapings, placental or fetal material—is necessary [26].

2.2. Manslaughter (other than VSA)

In the case of non-sexual assault manslaughter cases, contact between the victim and the perpetrator before death either does not occur—e.g., shot from a distance, explosion, poison—or only occurs for a relatively short period. In many cases, it is only indirect, where the perpetrator only goes to the crime location after the event to ensure that the act was successful and that the victim is indeed deceased. Generally, the perpetrator then leans over the victim and/or moves his/her body, during which head and body hairs, sweat, or epithelial cells from the perpetrator's hair, or fingers from where the victim was grasped can be deposited on the body of the victim [27]. In contrast to cases involving sexual violence, we most likely cannot count on discovering more kinds of biological traces or in any greater amounts.

In cases where the violence occurred using some form of equipment, it is necessary—and justified—to preserve samples from items found in, on or around the body, or embedded foreign materials, such as a knife, ammunition, shrapnel or splinters, etc., since it is plausible to attempt identification of the offender from biological samples that remain on these samples [28]. In the case of absence of traces of contact from the perpetrator, samples from the victim's clothing or found in the vicinity—e.g., animal hairs, particular leaves, plant compounds—are of increased importance since some of these may be deposited on the victim postmortem [29–32]. The DNA- or RNA-based examination of these environmental samples may lead to discovery of the primary site of the act in the case where the body has been moved [22, 33, 34]. If physical contact occurs prior to the manslaughter, e.g., a fight or scuffle, then a wider range of biological traces—for example, under the fingernails, epithelial cells at the point of gripping contact, or in the form of sweat, blood or hair—may occur [19, 35, 36]. The quantity of these found is, however, generally nominal.

2.3. Burnt human remains

In the event of home fires, automotive accidents, or disasters such as airplane crashes, industrial disasters accompanied by explosion, or explosive terrorist attacks, the bodies of the deceased have various degrees of burn damage to their tissues and there may also be severe fragmentation, decomposition, and intermixing of the remains of the victims [37, 38]. Rarely, in lucky cases, dental evaluation of intact skulls, examination of special dental implants—possibly containing a serial number, or a serial number on implants from other operations—may lead to the identification of the individual since, in many cases, the classic methods of identification, such as visual recognition, fingerprints, or facial recognition, do not provide usable results. In most cases, it is really only genetic tests which offer any hope of identifying body parts or the deceased individual themselves.

In those cases where the internal organs remain intact under the burnt exterior, genetic-based identification from soft tissue samples does not generally pose a problem. If the strength of the fire has been increased using fire accelerants or perhaps by the combustion of some highly combustible material in the vicinity and the bodies of the deceased have been carbonized, the soft tissue may have been destroyed and the bones severely burnt and more-or-less disintegrated. In this case, genetic analysis may be possible using the lesser burned areas and/or better preserved bones or teeth, although it is important to take into consideration during the sample-gathering process for nuclear or mitochondrial DNA analysis [39–41], the avoidance of areas of potential contamination which could preclude successful genetic examination, e.g., areas burnt with an accelerant, as well as to specifically locate gathering areas which may potentially contain usable information that is difficult or near impossible to visually detect.

Removing contaminants from the path taken by the biological remains from the scene to the laboratory is also essential since, especially fragile, the structurally compromised bone remains can be easily contaminated by contact with other individuals, such as members of the police force officially cooperating in the crime scene investigation, or those people involved in transporting the bones [42].

2.4. Immersed human remains

The identification of drowned bodies—bodies submerged by accident, suicide, or other reasons—such as traffic accident, or postmortem after a murder—that have entered water and sunk, and have decomposed, or are incomplete, is a complex task [43]. In the case of drowning, the typical autopsy findings that aid in determination of cause of death, due to the decomposition process, are mainly absent. In such cases, analysis of those water-dwelling single celled organisms—diatoms and algae—that enter the human bloodstream during drowning is justifiable. For this analysis, it is necessary to obtain samples from, primarily, the lungs or from other organs connected to systemic circulation. Bone marrow and spleen may also be suitable sources of samples for performing diatomic or blue algae specific morphological or genetic examinations [43–46].

Classic methods of identification are significantly limited in the case of bodies discovered that have been soaked for a considerable amount of time, and have started decomposing, have been partly or completely destroyed by possibly boats or driftwood, and are perhaps adipocerous. Visual recognition or facial database-based recognition is impossible, and recognition and identification based on scars from operations or tattoos can be problematic due to the state of the corpse. Examination of the clothing found on the corpse or foreign materials found on its body, for example, a scarf, belt, rope or other object around the neck or extremities, or items—or parts of items—discovered during the autopsy to be trapped inside the body, e.g., bullets, may provide valuable additional information. These may relate to the location that the body entered the water, e.g., the remains of a plant specific to a certain geographical area, or may aid in identification of the individual that used that item. Registered dental records, or serial numbers from special implants may also aid the identification process.

Depending on the length of the postmortem interval (PMI) [47], it would be justified to preserve muscle tissue, blood, hair samples, bone marrow, bone samples, and teeth for later identification of the corpse. These can be obtained using standard techniques during the autopsy. In cases of an aquatic context, the decomposition process may vary [48] and the effectiveness of the tests is significantly affected by the amount of time spent on soaking and in decomposition.

2.5. Highly degraded human bodies or body parts, and human skeletal remains

Recognition and identification of highly fragmented and/or degraded corpses and body parts discovered in advanced stages of decay as described above occur in numerous cases. External environmental conditions, such as climate, weather, and the geographical and geological conditions of the interment, biological components and characteristics of the given location, along with the amount of time the corpse has been exposed to these, play a decisive role in the decomposition processes following onset of death and in the degradation of the nuDNA and mtDNA contents of biological tissues. Due to long PMI, or the corpse being only partial, parts of or even all of it may have experienced significant damage, such as burning, explosion or chemical effects. The affected human soft tissues may be significantly degraded; however, bone and tooth samples can be subjected to and withstand harsher environmental conditions, e.g. ,exposed—burial, water immersion, and still produce varying levels of preservation [37, 49].

Human bones provide a relatively isolated and protected environment and can retain DNA for a long time without significant degradation, i.e., within bone cells, osteocytes, and those cells involved in rebuilding bones, osteoblasts and osteoclasts. The reason for this is that environmental effects—be it degrading enzymes, microorganisms, or ultraviolet rays—are not able to directly damage the DNA contained in the bone. Additionally, the material of the bone has a relatively low water content, meaning that the bone cells can easily dry out. In this case, their DNA bonds with the bone's primary building material such as hydroxylapatite crystals, acting as a protective environment, can preserve even larger sections for an extended period of time [36].

While the relatively suitable preservation of skeletal remains, including teeth, originally provided the main possibility for forensic age determination [50–52], the use of DNA-based molecular methods is currently being investigated [53]. Bone and teeth are the most often used human remains for performing identification using DNA analysis; but, in the case of RNA analysis, they can also be useful for determining PMI [49]. DNA can be isolated with varying degrees of success and effectiveness from different bones in the human body. In the case of a relatively short PMI and an intact corpse, tissues other than bone and teeth, such as blood, muscle, bone marrow, and brain matter, may also be sufficient for the performance of successful genetic analysis. Sometimes, there is even the possibility that instrument fragments or bullets [28] that either passed though the bone or embedded within the bone may preserve foreign DNA from the body of that person or animal that the device passed through before entering the victim's body. The examination of this type of mixed sample may be informative in connection with the circumstances surrounding the death. In the process of securing these samples, attention must be paid to specifying the primary and secondary sampling sites, avoiding contamination and standardization of sample storage [42].

2.6. Victims of animal attacks

Interaction and coexistence with animals may result not only in traumatic injuries, but may also have fatal consequences for humans [54–57]. The types of cases regarding a variety of exotic and wild animals as well as livestock or pets span a wide range from sharp force trauma to poisoning [58–63]. Animal attacks on humans occasionally may turn into lethal accidents, but usually the deaths resulted from unwitnessed attacks [63, 64] require medico-legal investigations. Autopsy evidences can determine the cause of death and reconstruct the dynamics of the fatal event in order to confirm that the attack was accidental and to exclude a mimic homicide [57, 63, 65].

In some cases, the cause of death is sharp and semisharp force trauma, which depends not only on the intensity of the trauma but also to a very great extent on the localization of the injury [65]. Animal biting wounds often permit a reconstruction of the perpetrator's teeth, but postmortem animal depredation may also cause such a bite pattern, which may initially suggest criminal assault [65], especially when animals, e.g., dogs, are enclosed in a home with a corpse [66–68]. Although bite marks can be discriminated from other traumas, bite mark analysis sometimes requires multidisciplinary approaches and should involve different forensic professionals [61, 69].

Although there are a large range of animals that have been involved in attacks on humans, pet dog attacks are relatively the most frequent type of case in medico-legal practice. Ordinarily, the injuries and consequences are related to the vulnerability of the victims, and children, the elderly, and disabled persons have the highest mortality rates [70]. Sometimes the victims of dog attacks are found naked, which can mimic a sexual assault rather than a dog mauling [61, 64]. Similarly to other violence cases, external examination of the corpse can reveal not only the relevant injuries, but also biological traces regarding to a putative perpetrator. Saliva or bitten material from biting area and animal hairs including those from the victims' clothing can be collected for DNA typing [64].

2.7. Collection devices and paraffin embedded tissues

Numerous relevant biological traces may be located on, or spread over the victim's body or in some body orifice. Naked-eye visual recognition and predictive identification of these potentially relevant—albeit in very minute quantity—remains might not be trivial and may require additional tools for preliminary tests. Inadequate or unnecessary sampling might be avoided through merely the technical application of non-invasive detection systems, such as special light sources, and the competence and professional expertise by the person doing the collecting, however, an all-encompassing combination of applied proficiency and practical common sense is necessary as well. Some methods can adversely affect the quality of, or reduce the quantity of retrieved DNA, while others may not pose a risk [71–78].

The application of a secondary transfer surface as a swab is a widely used and preferred protocol [26, 79–82]; however, taping—tape-lifting or stubbing—can be applied alternatively [83–85] for collection of even the epithelial cells of putative offender left on the victim's skin. Various types of swabs and tape are used [84, 86–89]. Retrieval of DNA depends on the sampling force—e.g., the pressure and actuation of the swab and contact intensity of adhesives—at the targeted area of devices and body, but may only partially pick up the available sample. This amount can be increased with multiple and coextracted swabs. In fact, the type of the biological material as well as the ratio of sampling area to the actual area of the deposit of relevant targeted remains, or interaction between victim's cells can influence the amount and mix of samples [89–91]. All this is relevant, even if the secondary transfer material is a liquid (buffer) in a device [92, 93].

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Although the contributing sources of DNA can be slightly modified with the careful, deliberate application of secondary devices, the majority of sample profiles usually can be obtained from the victim without trace component separation [82]. Molecular autopsy methods such as laser-capture microdissection (LCM) [94–97], flow cytometry, fluorescence (FACS) or magnetic (MACS) activated cell sorting methodologies [98–101] or alternatives [102–104] are fields where ongoing research is ensuring the potential to enhance the generation of single source genetic profiles of desired target DNA from cellular mixtures obtained by the initial collection device. Recent advances may provide a successful method for the separation of cellular mixtures of small numbers of cells [105], but complete separation of different cells with same morphology and gender origin seems to be a challenge that is still to be adequately addressed [98, 102].

The analysis of formalin-fixed, paraffin-embedded (FFPE) tissue can be essential when the legal process requires retrospective studies, for example, in criminal paternity testing, or cases of sudden unexplained death, or the paraffin blocks are the last and only option, e.g., identification of an unknown body, or for the purpose of determining a genetic profile [106–108], or even the cause and/or manner of death [109]. Formalin fixation process and subsequent storage is a perfect preservative for maintaining the integrity of tissues after death, but it causes crosslinking and degradation-fragmentation of the DNA over time, which prevents molecular PCR analysis and may cause difficulties in genotyping. DNA isolation from FFPE tissue is a decisive step toward a successful examination. Recent advances in technological developments have made generation of genetic information of DNA and RNA from these kinds of challenging samples possible [110–114].

3. Conclusions

Medico-legal autopsies have existed for thousand years, from the primordial period onward. Recently, the wide-ranging scale of procedures, alternative methods, and complementary examinations, such as forensic genetic analysis, are readily available and capable of being carried out for peculiar types of death [115]. Complex issues such as the determination of the postmortem interval, the identification of wound vitality, chronological reconstruction of injuries incurred, or the timing of natural diseases require interdisciplinary approaches and the involvement of multiple specialists, such as the forensic geneticist, among others [116]. In certain cases, the medico-legal autopsy is required by the courts to examine such fatalities in order to determine whether death is attributable to a homicide, suicide, animal attack, or a non-animal related accident, or perhaps due to some unidentified cause [57]. In cases of unknown bodies, skeletons or skeletal remains, as well as for victims of homicides or sexual violence, exemplary professionalism and utilization of appropriate sampling processes are equally important in perfecting the identification of both victims and/or perpetrators. As a primary principle of criminal law, an act must be concluded beyond a reasonable doubt, and a mere technicality, such as the improper preservation of samples or contamination of them, could effectively undermine the entirety of due process and could feasibly prevent a final verdict being made and subsequent sentencing against the true perpetrator of the crime. These aspects demand not only the usage of certified and accredited services and protocols, but also the comprehensive involvement of individual professionals in the field of necroscopic ascertainment—from anatomical dissection to experts in ancillary disciplines, such as the forensic geneticist—to ensure high standards of professional performance [116].

In regards to collection of DNA evidence during autopsy, standard protocols, DNA-free collection devices and appropriate methods of sampling, e.g., swabbings/scrapings/tape-lifting, must of course be taken into consideration along with the collection of trace evidence on the victim's clothing wherever possible. In these instances, application of less invasive methods, such as an alternative light source for identification of body fluids or epithelial cells for potential DNA testing is recommended.

The consideration of, and importance of contamination issues must not be neglected within the autopsy arena. The potential risk of DNA contamination in association with autopsy facilities, equipment, accessories—including impurities of body bags and autopsy tables—must not be ignored, and can be minimized not only by providing the proper protocols and equipment, but more importantly also by the application of personal professionalism [8, 27]. Despite increased attention, and due to the development of more and more sensitive DNA analysis, alternative scenarios are always represented, involving DNA transfer through a secondary person or medium. The minute amount of a few cells or DNA molecules, which may be applicable for amplification, is not recognizable typically in an autopsy environment and is easily transferable between cases [4, 117, 118]. Although methodological developments are continuously making efforts to eliminate the effect of contamination artifacts from the profiling process [119, 120], less appropriate autopsy sampling, incorporating the undetected contamination incidents, can lead to false interpretation within a DNA laboratory [5].

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