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# DNA Research in Forensic Dentistry

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**SUMMARY**

*DNA analysis has recently been introduced to forensic dentistry and is now frequently used in identifying individuals or determining the origin of certain tissues. This review reports on teeth and saliva as a source of DNA. Not only the quantity of DNA available for the laboratory is important, but also the quality and purity. Teeth are resistant against extreme circumstances such as temperature, humidity and acidity, which is an important advantage in DNA analysis. Furthermore, an abundance of DNA can be extracted from teeth. Saliva can be obtained in a single, painless and non-radical way. The double swab method is very effective; DNA recovery is significantly higher with the double swab method compared to the single swab or filter paper method. This review reports on the different techniques used to extract DNA from teeth and saliva, as well as DNA analysis of these samples. The usefulness and advantages of the double swab method for saliva, cryogenic grinding for teeth and chelex extraction and polymerase chain reaction for both types of samples is also described. DNA analysis has proven its value in forensic dentistry, but ethical and juridical considerations are still a matter of debate and criticism.* © 2001 Prous Science. All rights reserved.

**Key words:** DNA - Dentistry - Forensic

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## INTRODUCTION

In forensic science, various methods, such as the examination of external features, fingerprints and dental characteristics (including photographs and radiographs), can be used to identify individuals or to determine the origin of certain tissues. The use of DNA analysis has recently extended our possibilities in forensic dentistry. DNA can be isolated from many sources, as long as cells that contain an intact nucleus or mitochondria are present. This report will focus on teeth and saliva as a source. First, the structure and role of DNA will be described, as well as the existence of mitochondrial and cellular DNA, with special attention focused on the mutations that occur and the enzymes that are used in DNA research in forensic dentistry. The techniques used in DNA research in forensic science will also be reviewed, including sampling, extraction, quantification and analysis of DNA in samples obtained from teeth and saliva. The advantages of teeth and saliva as a source will be outlined together with the ethical and juridical aspects of DNA research in forensic sciences.

## DESOXYRIBONUCLEIC ACID

### Structure of DNA

DNA, a nucleic acid consisting of two complementary chains that are connected to each other through base pairs, is referred to as double strain DNA (ds DNA). Each strain is made up of nucleotides consisting of a sugar (desoxyribose), a base and phosphoric acid. There are two kinds of bases: purines (adenine, A and guanine, G) and pyrimidines (cytosine, C and thymidine, T in DNA or uracil, U in RNA). Each base binds to its specific counterpart in the second chain (A to T, C to G) through base pair formation (AT, CG). Each separate chain has a 3’ and 5’ end indicating the orientation of the sugars in the chain. The two chains are wound around each other and form a double helix (Fig. 1). In combination with histones and acidic and basic proteins, DNA is condensed to a chromosome (Fig. 2). In normal cells, a diploid number of chromosomes are present (46 in human cells), while in human reproductive cells this is a haploid number (23). Diploidy is the appearance of chromosomes in pairs (2 x 23 in humans); Within a pair, one chromosome is from a spermatozome and one from an ovary cell (1, 2).

### Role of DNA

DNA can be considered as a sequence of bases that is present twice —once in each complementary strain. The information in these sequences is coded. Three mononucleotides (each consisting of a phosphate, a sugar and a base) form the code for a particular amino acid, which is the basis of proteins. The sequence, the number and the kind of amino acids are characteristic for the properties

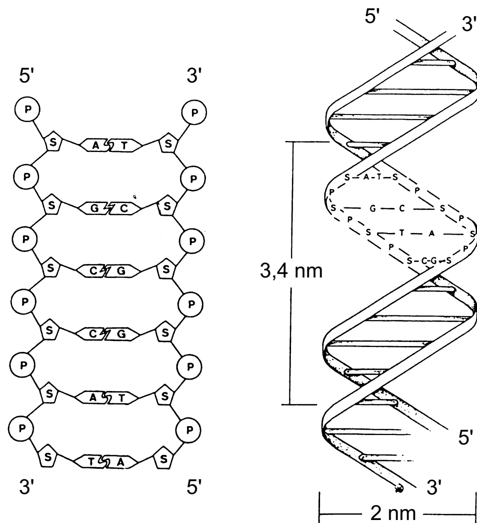


FIG. 1. Structure of DNA. A: adenine; C: cytosine; G: guanine; P: phosphoric acid; S: sugar (desoxyribose). (Reproduced with permission from Cokelaere, M., Craeynest, P. Onze Genen, Handboek Menselijke Erfelijkheid. ACCO 2000.)

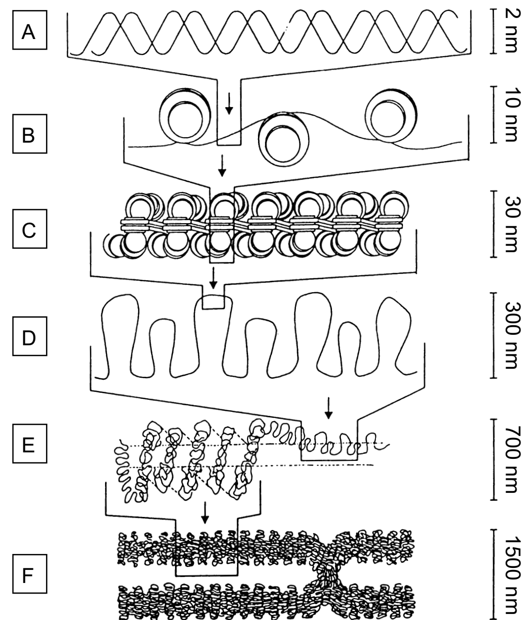


FIG. 2. Phases in the conversion of a DNA chain to a chromatid of a chromosome. A) Light spiraling of the double DNA chain; B) DNA chain winds around histones forming nucleosomes; C) The fiber winds around itself; D) and E) The thick fiber forms loops around a protein core and spirals further to a chromatid; F) The two chromatids are connected at the centromere and form a chromosome. (Reproduced with permission from Cokelaere, M., Craeynest, P. Onze Genen, Handboek Menselijke Erfelijkheid. ACCO 2000.)

of a particular protein. The sequence of amino acids is exactly coded in DNA. An accurate synthesis of proteins occurs from this code to the final protein. A few copies of the piece of DNA that contains the particular code are first made in the nucleus of the cell; this is referred to as transcription of DNA to RNA. These copies contain more data than necessary for the synthesis of a protein. After transcription, the primary RNA (pre-RNA) is purified and adapted to functional or mature RNA. The mature RNA leaves the nucleus and in the cytoplasm the ribosomes take care of the attraction and attachment of the exact amino acids, using the information fixed in DNA and present as a copy in the RNA. This conversion of RNA to protein is called translation (Fig. 3).

Proteins perform diverse functions in the body: structural proteins serve as components of organs; transport proteins bind and transport certain substances (*e.g.*, hemoglobin transports oxygen in the blood); protective proteins serve for the defense of the body (*e.g.*, antibodies) and regulatory proteins drive certain processes (such as cell division). The most important, however, are the enzymes that control the biochemical processes in the body.

The sequence of bases containing the code for a particular protein is called a gene, which is localized at a specific place on the chromosome, namely the locus. This code is present in each chromosome of a chromosome pair. Various genes have different variants, called alleles, which are present at the same locus and contain information about the same proteins.

DNA is thus important for the synthesis of proteins and contains genetic information. This information is

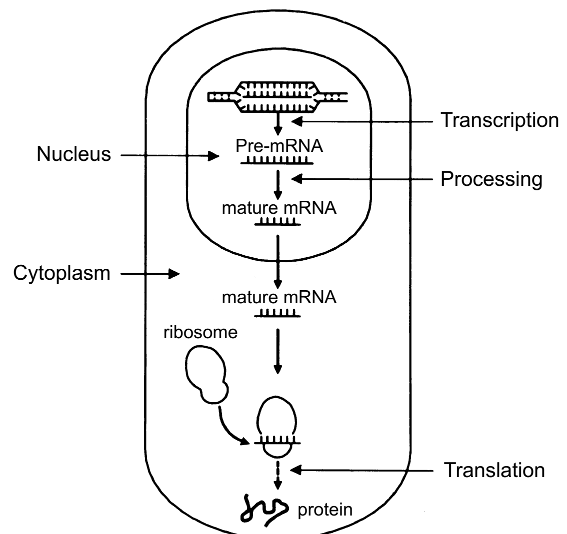


FIG. 3. Steps in the conversion of DNA to RNA and to proteins. Reproduced with permission from Cokelaere, M., Craeynest, P. Onze Genen, Handboek Menselijke Erfelijkheid. ACCO 2000.

situated in 10% of the base pairs. The remaining 90% is noncoding or junk DNA. These zones show a high degree of variation in sequences between various persons

(1). A complete DNA chart can be drawn, but it is too cumbersome to determine a complete DNA chart for each person.

Therefore, certain regions where the variability between persons is sufficiently high are considered. This diminishes the chance of two persons having the same sequence in that area. These polymorf regions are repetitive and are made up of many repeated units of the same base sequences called variable number of tandem repeats (VNTRs) or satellite DNA.

Depending on their length, VNTRs are divided into 3 groups: i) classical satellite DNA (clusters of DNA sequences with a length of 100 to 500 kilobase (kb) or 1000 base pairs); ii) minisatellite DNA (sequences of 100 to 20,000 base pairs) and iii) microsatellite DNA (cluster of less than 100 base pairs, called short tandem reprints, STRs) (1, 3).

**Cellular and mitochondrial DNA**

The majority of DNA—cellular DNA—is present in the cell nucleus as chromosomes. A small fraction, however, is present in the mitochondria. Each mitochondrion contains 5 to 10 ring-shaped pieces of DNA, called mitochondrial DNA (mt DNA), which are important for the structure and function of the mitochondrion. A child inherits its mt DNA only from the mother. The fertilized ovary cells only contain mitochondria from the cytoplasm of the ovary cell. The mitochondria of the spermatozome are located in the tail, which breaks off when the head of the spermatozome penetrates the ovary cell at fertilization. The advantages of mt DNA in forensic dentistry include the following (4): i) identification is possible by comparison with any family member connected with the mother; ii) mt DNA is haploid, and thus easier to handle and work with; iii) there are a higher number of copies present and various mitochondria per cell; and iv) mt DNA contains high polymorf regions, which augments the value of the test, as these regions contain a high number of variations in the DNA sequence, and thus decrease the possibility of resemblance between the donor and volunteer.

## Working with DNA

*Mutations*

A mutation is a sudden change in the structure of DNA that can occur spontaneously or be elicited by external factors. There are four kinds of gene mutations: i) point mutations (one base is replaced by another); ii) insertions (one or more base pairs are added); iii) deletions (one or more base pairs are lost); and iv) inversions (a piece of DNA is liberated and inserted at the same place but in reversed sequence).

If such a change occurs in a piece of DNA that encodes a protein, then the protein and its function will be different. The consequences are often a diverged action in the organism and can even be life threatening. Although changes in junk DNA usually do not result in visible consequences, they are important in forensic science. These mutations serve as pronounced variations between individuals.

*Polymerase chain reaction*

The polymerase chain reaction (PCR) is a technique used in DNA analysis in which the amount of available DNA can be multiplied. PCR is based on a cycle of temperature differences. First, a rise in temperature induces, at the melting point, a denaturation of DNA and the two strains are uncoupled from each other. In the second step, the temperature is lowered in the presence of an excess of sequence specific primers. Primers are small pieces of polynucleotides that bind at a specific locus to the single-stranded DNA-fragment to indicate where polymerase can start. The binding occurs through hybridization whereby two single strains have the tendency to bind to each other. The more complementary these strains are, the more stable this binding is. The primers are then extended by DNA-polymerase. This polymerase makes the complementary strain against the first chain and starts at a piece of double-stranded DNA. The two separate chains are both completed until they both have their own complementary strain. The result is that there is now twice the ds DNA than there was at the beginning. This cycle is repeated until the desired amount is obtained (5).

The major advantage of this technique is that even a small amount of DNA can be multiplied and used for analysis (1). It is, however, advisable not to repeat the cycle indefinitely, as the purpose is to amplify the targets and not to possibly contaminate the material. The latter will only be a small part of the total sample, but if it is amplified too much it will start playing a significant role (6).

DNA-polymerase completes an unfinished double chain in the 5’ direction (Fig. 1). Polymerase from *E. coli* was previously used, but it is not temperature stable and has to be re-added at each cycle. A polymerase from *Thermus aquaticus* (Taq) has been recently used; this bacteria lives in hot sources and its polymerase is stable. Therefore the enzyme is added only once for the various cycles (5).

*DNA research*

DNA can be split by restriction enzymes, which cut DNA at certain loci. DNA ligase binds the various parts together again.

DNA is first extracted from the cells obtained from saliva or teeth, and then centrifuged to separate the DNA from the other cell components. After cutting by a restriction enzyme, the fragments are separated by gel electrophoresis. DNA has a negative charge and will migrate in an electric field to the positive pool. If this

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occurs in a gel, not only the charge and form is important for the extent of the migration, but also the size. During the same time period, the short pieces will migrate over a greater distance than the long pieces, because they have less resistance. This technique can thus be used to separate the DNA-fragments according to their size.

After separation, the DNA research can be completed. Using probes, marked pieces of DNA or RNA go through a process called blotting. After denaturation, a probe with complementary segments for certain specific sequences is added. These segments bind the single-stranded chain (hybridization), and make them visible, due to the radioactive marking.

## DNA RESEARCH IN TEETH AND SALIVA

DNA can be isolated from various sources, as long as they contain nucleated cells, as every somatic cell contains the whole genome (1). Such sources are blood, semen, tissues, organs, bones, hair, nails, teeth, saliva, urine and other body fluids (7).

### Techniques for DNA research in teeth

*Collection of samples*

Teeth have a rich supply of genetic information. Nucleated cells can be isolated from the surrounding bone, periodontal fibers and blood. However, the chance of contamination or degradation is very large, except in the pulp (Fig. 4) (8).

The pulp consists of odontoblasts, fibroblasts, endothelial cells, nerve tissue, mesenchymal cells and blood cells. This cellular component (present in the pulp chamber and in the roots) decreases with age (9).

*Crushing*. One way of collecting DNA from teeth is crushing, which is the grinding of complete teeth. Although a lot of DNA can be obtained with this technique, the morphology of the teeth together with the restorations are lost (9). The latter are, however, a useful source of information, *e.g.*, for radiologic or morphologic comparison.

*Conventional endodontic access.* It is difficult to obtain enough DNA through conventional endodontic access. In addition, the occlusal morphology and the restorations are damaged (8).

*Vertical splitting*. A lot of pulp tissue can be obtained with the vertical splitting method, although the restorations and the tooth are also damaged. However, this technique is practically impossible due to the various forms of the roots (9).

*Horizontal section.* A fourth possible approach is the horizontal section, in which the tooth is split at the enamel-dentine border. The access of the pulpa room and the roots is sufficient and also the crown remains intact (8).

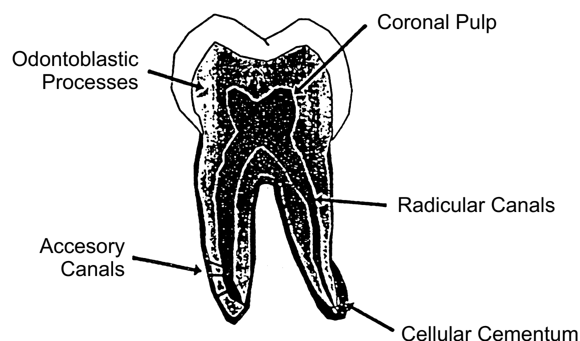


FIG. 4. Places in the teeth where DNA is present. No DNA is present in the enamel. (Reprinted with permission from Smith, et al. *A systematic approach to the sampling of dental DNA*. J Forensic Sci 1993, 38: 1194-209. ©ASTM.)

*Cryogenic grinding*. In cryogenic grinding, the tooth is frozen as a whole using liquid nitrogen and put in an electromagnetic room. The tooth is ground to a fine powder by alternating magnetic fields (10).

*Discussion*

Crushing seems to give better results than sectioning of teeth as more DNA can be obtained. The higher chance of DNA damage in this technique is not important considering the sensitivity of PCR. The recently developed method of cryogenic grinding is easier and very effective. It also allows to obtain DNA from endodontic treated teeth because of the presence of DNA in hard tissues (10).

### Techniques for DNA research in saliva

Saliva can be taken directly from the mouth or collected after deposition on the skin (through biting, sucking, licking). Saliva can also be found on objects such as post stamps, envelopes and cigarette ends (11).

A fresh sample from the mouth can be obtained through the collection of saliva or by a buccal swab. With a dry swab one rubs softly over the inner surface of the cheek. This buccal swab is then dried at room temperature (11).

Saliva on the skin can be obtained by a single swab with a sterile wet tissue, by a wet filter paper placed on the skin (reduction of possible contamination) or by a double swab technique (12). In the latter technique the skin is first gently rubbed with a wet, sterile swab, whereby a rehydratation and a release of the present cells occur. Then the cells are collected with a second dry swab. Both swabs are collected into one sample (12).

All samples obtained with the three techniques are dried at room temperature and stored at 4 °C. A

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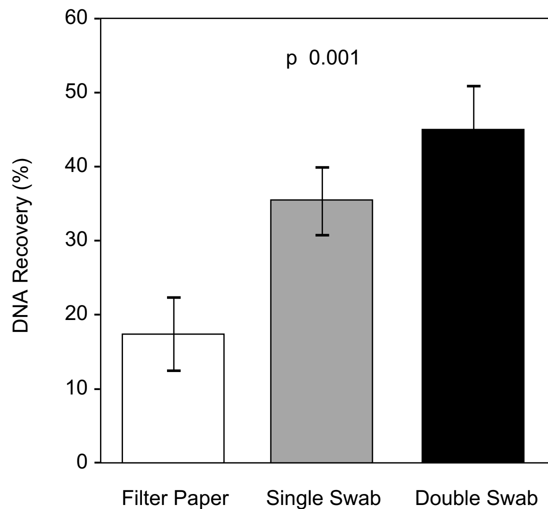


FIG. 5. Comparison between the various methods used to isolate DNA from saliva on the skin. (Reprinted with permission from SSweet, D et al. *A systematic approach to the sampling of dental DNA.* J Forensic Sci 1997, 42: 320-2). ©ASTM.

comparison between the three methods (Fig. 5) shows that the double swab technique gives the highest DNA recovery (44.6 ± 6.4%) versus the filter paper method (17.4 ± 5.0% DNA) or the single swab method (35.3 ± 4.8%) (12).

Saliva found on objects can also be collected. After drying at room temperature, the object (*e.g.*, post stamp, cigarette end) is cut into pieces and put into a buffer solution (12). All samples are stored in a sterile refrigerated container. These containers are closed, labeled and transported to the laboratory as soon as possible (7).

**DNA extraction**

DNA extraction allows the release of cells from the substrate arriving in the lab and the liberation of DNA from the cells.

DNA extraction from dental material consists of dissolving the material in a buffer solution (guanidine-EDTA), followed by centrifugation. The white precipitate is again dissolved in buffer, proteinase K is added and the solution is incubated overnight. The final step consists of a second centrifugation step plus the inactivation of proteinase K (13).

DNA extraction from saliva can be performed by an organic method. After dissolving the material in a buffer solution, centrifugation and proteinase K treatment, fenol-chloroform is added (14). The disadvantages of this method are the use of toxic substances and the fact that it is time-consuming (15).

Instead of fenol-chloroform, the saliva samples can also be extracted by chelex-100. The metal ions are then chelated and inhibited. This prevents the degradation of the samples by its warming, which is necessary for the release of the cells from the swabs. Chelex also removes the impurities present in the material. This method is simple and fast (14).

A modified Chelex method is also used. The sample undergoes a preextraction. It is first warmed to release the cells from the swabs, boiled without chelex to eliminate the nucleases and then boiled in the presence of chelex to extract the DNA.

The advantages of the Chelex method over the organic method are that there is higher DNA recovery, no use of toxic products and it is less time-consuming.

The modified Chelex method increases the number of released cells and the amount of available DNA, but it is more time consuming than the classical Chelex method. The modified Chelex method is therefore especially used when there is little DNA present in the samples (*e.g.,* dried-up saliva on the skin) (6, 14, 15).

*Slot Blot quantification*

The quantity and quality of the extracted DNA is checked by Slot Blot (16). Only a small part of the sample is used. DNA is denaturated by the addition of NaOH, the solution is applied to a nylon membrane in a Slot Blot apparatus and fixation is obtained by UV light or heating. After prehybridization a probe is added with high repetitive, primate-specific DNA fragments. These sequences are marked and, after attachment to the denaturated DNA strands, are made visible by autoradiography. The human origin of the obtained DNA can be demonstrated in this way.

**DNA analysis**

*Restriction fragment length polymorphism*

In the restriction fragment length polymorphism (RFLP) method, the DNA strands are cut in fragments by a restriction enzyme. These fragments are electrophoresed on an agarose gel, transferred to a nylon membrane and incubated with a radioactive probe, which contains complementary pieces of polynucleotides that hybridize with the fragments and mark the various parts.

Two kinds of genetic variation can be detected in this way. First, a mutation in the zone where the restriction enzyme attacks the strand, a place that is normally cut, is not recognized. The two pieces remain together forming a long piece that has more resistance against migration in the gel compared to the two pieces separately. The second variation is when the distance between two restriction enzymes alternate through insertion, deletion or variation in a number of repeating units. This is particularly useful in forensic science. The human genome contains zones of VNTRs in many places. The variation in repeating units will determine the length of the fragments between two cutting loci.

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Restriction fragment length polymorphism has limitations, however. Sufficiently high molecular DNA is necessary and it is also a cumbersome method (8).

*Polymerase chain reaction*

Polymerase chain reaction (PCR) can be used for DNA analysis in two different ways: the amplification of polymorf regions and the determination of the variation in their sequence or selective multiplication of an allele by a specific primer.

The PCR products in the first proceeding can be investigated in three ways. The first involves hybridization with marked sequence specific probes. After binding to the PCR products, which are separated on a nylon membrane, certain sequences are made visible if they are present.

A second way is electrophoretic analysis of the PCR products. The sample is put on a membrane and the different pieces will migrate over another distance under the applied tension, because different lengths (obtained by insertion, deletion of repeating units) lead to different resistances. This method is described as the analysis of amplified fragment length polymorphism (Amp-FLPs).

The third way is through determination of the proper DNA-sequence of the PCR-product, which is more time-consuming.

In the selective amplification of an allele by a specific primer, there are two different primers for two alleles. If one primer is added and the other allele is present in the sample, there will be a mismatch at the 3' end and no prolongation can occur for PCR. In this way an analysis can be performed that gives a different result for each individual.

Identification can then be performed by comparison of the patterns obtained from analysis of the test sample and of the known sample. The advantages of PCR-based DNA analysis are that its sensitive and specific, less time consuming, less extensive and no radioactive markers are necessary (5, 17, 18).

## BITE MARKS

Bite marks can be used for physical comparison, although this is not easy. In the first place, the use of bite marks is subjective and dependent on the experience of the investigator. There is also always deformation because the skin is elastic. A bite mark can also be seen in various forms.

The American Board of Forensic Odontology (ABFO) makes a distinction between types of lesions (*i.e.,* erythema, contusion, abrasion, laceration, incision and avulsions [22]). Saliva can also be transferred to the skin by bites. These bite marks can be used as biological proof or evidence because DNA can be isolated (15, 16).

Aside from bite marks on the skin, it is always possible to try to obtain DNA from other bite marks, *e.g.,* in food as described by Sweet and Hildebrand (23) in a bite mark in cheese.

## DISCUSSION

### Teeth as source of information

Tooth enamel is a very strong material. Teeth are normally resistant against extreme circumstances, and therefore, their morphology is well preserved. Not only the external form is preserved, but the enamel also protects the content of the pulpa chamber against external factors. This is very useful in forensic dentistry, because identification is possible by comparison with antemortem records (19). Moreover, hydroxyapatite, an important component of dental tissue, binds to DNA and stabilizes it (4).

Teeth are thus very suitable for DNA isolation. It is proven that dental tissue is resistant against factors such as water, temperature rise, high and low pH, salt water and aging. An abundance of DNA can be extracted from teeth because of dehydration, which prevents eventual putrefaction (20).

### Saliva as source of information

Saliva is deposited on the skin by kissing, licking, sucking, biting, *etc*. Previously, identification tests were performed by determining blood group, antigens, proteins or isoenzymes. Because of their low concentrations, these methods were less sensitive (21).

Another way is to collect the cells present in saliva and analyze their DNA. Such cells are called oral epithelial cells and leukocytes (9). It is important to use a technique that provides enough DNA and that contamination with the DNA of skin cells does not occur.

As mentioned previously, the double swab method is very effective. Sweet *et al*. (6) demonstrated that no contamination occurred with this method. When the double swab method was applied to skin without the presence of saliva (as a negative control), DNA was not detected by Slot Blot quantification (6).

The quantity of useful DNA obtained in a saliva sample with the double swab method decreases with time, especially during the first 24 hours after deposition to the skin. Possible explanations for this are a potential adhesion between the cells from saliva and epidermis and a certain degree of degradation of the cells (6).

### Juridical value

In the case of a juridical DNA investigation, a DNA expert is appointed by a magistrate of justice. This expert analyzes the available biological samples. If two profiles are different, a person can be excluded with certainty. If, however, two identical DNA sequences are found, a statistical approach has to be made: what is the frequency of a particular sequence at a specific locus in that population?

There are often discussions about the results and their value (24). Criticism was first directed to the procedures in the laboratory, but after improvement of these

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procedures the criticism is now directed more to the statistical approach.

**Ethical consideration**

The controversial arguments surrounding the ethical responsibility of DNA analysis can be reduced to two issues (24): i) human values (privacy, respect for the body, *etc*.) are threatened by constructing a DNA databank. On the other hand, human values (self-defense, lock-up of criminals, *etc*.) are also threatened by not making such a databank; and ii) by maximizing the identification of criminals, one also increases the chance that an innocent person will be convicted. However, if this is not done, the chance that a criminal goes free from justice also increases.

According to Beyleveld (24), taking into account the rights of the humans as recorded by the European Convention on Human Rights, it is worse to lock-up an innocent person than to release a criminal.

**Situation in Belgium**

DNA analysis in Belgium occurs in university laboratories and since 1992 in the NICC (Nationaal Instituut voor Criminologie en Criminalistiek), Brussels, Belgium. There is also a Disaster Victim Identification team (DVI) of the federal police. This team collaborates with NICC and, if necessary, an expert in DNA research is appointed to the team. The directives for these investigations are determined by the European Community (25).

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