# **Molecular Analyses**

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## Chapter 5

Forensic DNA Samples: Collection and Handling

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## 5 Forensic DNA Samples Collection and Handling

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#### **INTRODUCTION**

Under dry and cool conditions, but sometimes even at temperatures over 50°C, DNA is quite a stable molecule (e.g., on paper, in hair, as skin flakes, blood, saliva and sperm, from old fingerprints, and out of bones and teeth<sup>[1–12]</sup>) in induced anhydrobiosis utilizing storage matrices like Samplematrix<sup>TM</sup>,<sup>[13,14]</sup> or DNAstable<sup>TM</sup> plates, but also home-(lab-)made trehalose and polyvinyl alcohol (PVA) plates.<sup>[8]</sup> DNA in epithelial cells can survive soapy water.<sup>[15,16]</sup> Under indoor conditions, 10 minutes rinsing of clothes under the tap, or one week in the bathtub, skin cell DNA can be recovered from clothing. Outdoors, temperature and movement of water (pond vs. river) are of

relevance. Complete STR (short tandem repeats) profiles were found after two weeks in a sample of skin cells in a pond during cold winter vs. only 4 hours in hot summer conditions.<sup>[17,18]</sup> Domestic machine washing and drying often leads to transfer of foreign DNA onto freshly laundered items. After swabbing, around 1 ng of foreign DNA was found ('indirect' or 'innocent' transfer). In most cases, the wearer is the major or sole contributor,<sup>[19–21]</sup> however, interpretation of such stains is a delicate matter. On semen-stained UK school uniforms (T-shirts, trousers, tights) that were stored in a wardrobe for eight months,  $6-18 \mu g$  of DNA were found after washing them multiply at 30°C or 60°C. Unstained socks that had been washed together with the stained items contained one-tenth of the sperm donor's DNA.<sup>[22]</sup> Spermatozoa persist on cotton and terry towels for at least six wash cycles.<sup>[15]</sup> Vaginal secretions also leave amounts of DNA on clothing laundered at 30°C that are sufficient to produce complete genetic profiles.<sup>[23,24]</sup>

From a technical and criminalistic point of view, DNA can be collected and stored like most visible biological stains. Crucial considerations in the examination of evidence include photographic documentation, and careful storage of the samples under dry and cool conditions. Special aids such as sexual assault kits, swabs, drying devices, and filter paper treated with denaturants are available and should be used. However, DNA collection in forensic environments is not a merely technical but also a criminalistic task. Two questions are of special importance: (1) whether a stain is of relevance to the actual crime, e.g., if it could have been left at the scene some time ago by persons who are not related to the crime, and (2) if a stain should be used for extraction straight away, or stored as long as possible for morphological measurements and crime (scene) reconstruction, e.g., the form of blood stains on wallpaper, the exact location of sperm stains on clothing, or the exact location of skin cells found on furniture.

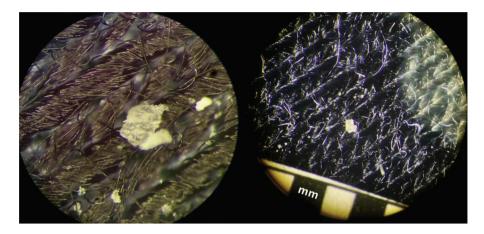
#### **EVIDENCE EXAMINATION**

Irrespective of possible chain of custody rules, examination of evidence starts with photographic and/or drawn descriptions of the items received by the forensic biologist. In every photograph, an absolute scale must be visible (millimeters/centimeters; no pennies, no pens). Resolution should be  $\geq$ 3266 × 2449 pixels = 8 MPixel to allow blowing up of the pictures. Since this size is now easily achieved by most cell phones, these phones may be used after appropriate training. They also work for microscopic photographs (Figure 5.1). Use of a flash should be avoided because brighter parts of the objects often 'flash out' (become white). Biological stains that were detected either by their surface properties (detection by touch: e.g., sperm stains on dark clothing),<sup>[25]</sup> monochromatic light (e.g., saliva), or regular bright light (e.g., hair or small blood stains) are circled and numbered by use of a water-resistant pen or neon color.

#### **COLLECTION OF BIOLOGICAL STAINS**

#### **S**WABS

Practically all stains can be collected by rubbing them off with a cotton swab.<sup>[26,27]</sup> Stains on fabric should be cut out first (Figure 5.2). Swabs are soaked with one drop of fresh distilled sterile water. After transfer of the stain to the swabs, they must be



**FIGURE 5.1** Freehand micrographs captured during initial examination of a mask. The micrographs were captured using an iPhone 11 Pro and dissecting microscope (Leica Mz 12.5 at magnifications 25× and 100×, respectively).



FIGURE 5.2 Removal of stains by cutting stained materials.



FIGURE 5.3 Swab collection tubes.

dried immediately.<sup>[28]</sup> DNA sampling tools that offer rapid drying can significantly improve the preservation of DNA collected on a swab, increasing the quantity of DNA available for subsequent analysis. In saliva samples, slow drying of swabs in storage tubes leads to a decrease from a yield of 95% recoverable DNA to only 12% recoverable DNA.

After collection, the swabs remain inside of their tubes and are put in protective containers (Figure 5.3). A convenient way to dry swabs is to put them into a closed cardboard box at room temperature (Figure 5.4). There, they can neither touch neighboring objects nor develop mold.<sup>[29–32]</sup> Swab tubes consisting of a paper wall stabilized with plastic are preferable since nothing has to be assembled, i.e., the risk for contamination is lowered. Cardboard boxes are a good place to store evidence because residual moisture, especially from clothing, can easily evaporate (Figure 5.5). Touched objects may be rubbed with cotton tips moistened with a 2% SDS (sodium dodecyl sulfate) solution.<sup>[33]</sup>

In professional forensic environments, contamination caused by airflow during the drying process has not been reported to be a problem. Some field laboratory manuals ask for drying in closed cupboards (Figure 5.6) or under sterile laminar airflow. If used, cupboards must never be tightly closed to avoid building up of humidity and mold. If minute amounts of DNA, especially mtDNA or Y-chromosomal DNA may be of relevance, freestanding cupboard drying must be avoided. Under field conditions in poor countries, it is still an option when only STRs are used.

Swabbing is performed by *intense, multiple* rubbing of the stained surface to collect a maximal amount of DNA. Inside of oral cavities, the cotton swab is rubbed against the mucous membrane; saliva alone may not contain enough cells. After







**FIGURE 5.5** Samples are wrapped in paper ready for transport or storage.



**FIGURE 5.6** Under extreme field conditions, cupboards may be used for drying of swabs. Contamination is not a problem as long as the swabs do not touch each other or the wall. Note that the cupboard must not be tightly closed to avoid building up of humidity.

complex shooting situations, used bullets can be matched to the victims by swabbing off traces of tissue that remain on the bullet once it enters the body.<sup>[34]</sup>

#### **EARLY SWABBING**

Swabbing of clothing items, especially of skin, should be performed as soon as possible in forensic and police investigations. For example, DNA typing was possible in the following cases where swabs had been collected early at the scene of the crime. Before swabbing, intelligent criminalistic assumptions concerning the location of the invisible yet possible stains had been made. The swabs may be made of cotton or synthetic material.<sup>[35]</sup> They must be thoroughly checked to be DNA-free in the laboratory, because in some circumstances, they may become contaminated. In a very severe German serial homicide case, contamination misled the prosecution and police for years – the alleged culprit was an old woman working in the (German) cotton swab factory who had touched the material at times.<sup>[36]</sup>

In contrast to common belief, corneocytes contain DNA. Therefore, all surfaces that may have been touched by an offender (through grabbing of ropes, wearing of baseball caps, hitting a person, inside of gloves) may be swabbed (or lifted, see 'Touch DNA') successfully.<sup>[37–39]</sup> Epithelial cells of an unknown suspect were swabbed off the front side of a collar of a polo-neck pullover. The victim had been

stabbed, but the stains had not been visible on the collar.<sup>[40]</sup> Alternatively, single cells can be observed microscopically, taken off either with a pair of forceps or a vacuum device, and then used for single-flake amplification.<sup>[12,41]</sup> DNA contained in epithelial cells that had been transferred by saliva of an offender was swabbed off the skin of an experimental victim that had showered. Amplification of the offender's STRs and Y haplotype was successful up to several hours after transfer of his saliva to the skin of the victim.<sup>[42]</sup>

Early swabbing is also necessary whenever cells from the top edge of bottles, beer cans, etc. are collected. Collection of the complete bottle or can frequently leads to spilling of its contents and dilution or washing off the cells. If early swabbing is not possible, the liquid must be drained out of the container by drilling a hole in its bottom.

#### **DOUBLE SWABBING**

Double swabbing is the use of a wet cotton swabs first followed by rubbing with a dry one. It often leads to better results in cases of touched objects (sweat) and bite marks (saliva).<sup>[43,44]</sup> After double swabbing, around 1/5 of the expected alleles can be amplified even from bullet cartridges that have been fired – a particularly challenging surface.<sup>[45]</sup>

#### FILTER PAPER

Liquid blood can be stored on filter paper that is then dried in the same way as cotton swabs (Figure 5.4). Filter paper that contains denaturants, buffer, and a free radical trap (e.g., FTA paper<sup>TM</sup>)<sup>[2,46,47]</sup> will lyse the blood cells and immediately deactivate blood-borne pathogens such as herpes, cytomegalovirus, and HIV. Filter paper can also be used to store saliva and liquids from decomposed bodies, especially tissue (cells) from internal organs.<sup>[48]</sup> If the DNA is too degraded, regular STRs may be subsequently substituted by massive parallel sequencing (MPS). This allows detections of numerous single nucleotide polymorphisms, which are suitable for identification of body parts, for example.<sup>[49]</sup>

In automated laboratories, standard-sized filter paper is the preferred option. Pieces can easily be punched out of it by a machine and subsequently processed by a DNA extraction and polymerase chain reaction (PCR) robot. The advantage of FTA paper over regular filter paper is that it can be used for multiple PCR reactions. Template DNA will stick to FTA paper after washing off the PCR products and can then be reused. It is also suitable for touch DNA. On steering wheels, FTA paper collects a two-fold amount of DNA compared to double swabbing or tape lifting.<sup>[50]</sup>

#### **ELECTROSTATIC SAMPLING**

For the sampling of trace DNA from clothing, electrostatic dust print lifters (DPL) have the same success rate as sampling with wet cotton swabs. However, in single

aggressor cases, almost no mixed aggressor-victim profiles suitable for database entry can be established, which is sometimes necessary to better understand the case criminalistically.<sup>[51]</sup>

#### URINE AND FECES

Because feces are found especially at scenes of (serial) burglaries, it should be collected irrespective of its repulsive nature. Fresh feces as well as liquid urine should be frozen below  $-20^{\circ}$ C to avoid bacterial activity. DNA typing of urine is successful especially if it was excreted in the morning (when the highest number of epithelial cells are found compared to the rest of the day)<sup>[33,52]</sup> and from feces after PCR inhibitors are removed. To recover the cells, urine needs to be centrifuged (cells are located in the sediment), whereas stool samples can be extracted straightaway or from swabbing with mini spin columns. The estimated number of up to  $6 \times 10^5$  pg human DNA/mg stool is never reached in practice because of bacterial and digestive action. Nevertheless, up to 170 pg DNA/mg stool were successfully extracted and amplified under case work conditions.<sup>[53,54]</sup>

#### SEXUAL ASSAULT KITS

After sexual assaults, biological stains are often collected in a hospital environment, at home, at a general practitioner's office, or at a police station. To avoid contamination of the samples and to allow full collection following a checklist, sexual assault kits are available. Their use is generally and strongly recommended to guarantee collection of all stains in the best possible way even under highly stressful conditions or in cases where lay personnel have to collect the evidence.<sup>[27]</sup> The kits consist of prepacked envelopes in a cardboard box, which can be stored and stacked at room temperature (e.g., Sexual Assault Care Kit, University of Bern, Figure 5.7). The envelopes contain swabs, combs for hair (head and pubic), filter paper, sterile distilled water ampoules, large paper bags, and standardized protocol sheets.<sup>[30]</sup>

#### CLASSIC FINGERPRINTS AND 'TOUCH DNA'

In fingerprints, the DNA loss ranges from half to three quarters of the DNA compared to the amount of cells transferred during the touch event.<sup>[55,56]</sup> However, DNA is resistant to many histological stains, including substances used to develop fingerprints (or other skin lines). DNA typing was successful from developed skin line prints after cyanoacrylate (super glue fume) or color reagents such as amido black, leucomalachite green, Hungarian Red, DFO, or luminol had been applied.<sup>[57–60]</sup>

Developed skin line prints should first be documented with a high-resolution camera. The original skin line prints can then be submitted to DNA storage and extraction like any other biological stain. The stronger the initial fingerprint or 'touch', the more likely a DNA profile may be obtained.<sup>[11,61–63]</sup>



**FIGURE 5.7** Sexual assault kit: Standardized descriptions and checklists are printed on the actual envelopes that contain the collection materials.

Since humans shed around 400,000 skin cells daily, it takes only two seconds of handling time to transfer enough DNA ('touch DNA') onto clothing to obtain a complete profile.<sup>[64–67]</sup> Full DNA profiles can be obtained from the cells of a person sleeping in a bed for a night or after ten minutes of wearing a sweatband.<sup>[68,69]</sup> During wearing for a day, the DNA quantity – mostly DNA from the wearer of a shirt – increases eightfold. On the back area of a shirt, DNA mixtures are more likely than on the front.<sup>[18]</sup>

When targeting for such trace DNA, the sample area should be narrowed down as much as possible to maximize target DNA recovery (Figure 5.8).<sup>[66,70,71]</sup> Care must be taken not to destroy the evidence during such narrowing down because any type of air movement or vibration (tram tracks, laboratory equipment) as well as static electricity that builds up between plastic forceps and some types of garment may cause skin flakes to jump out of sight.<sup>[72]</sup> If no skin particles are visible, small segments of the garment should be either rubbed, taped, or used as a whole to avoid DNA mixtures from neighboring areas. The same is true for fingernail clippings where each nail clipping might be cut down into thin segments, each for one PCR tube.

Swabbing of 'touch DNA' is often successful as well as direct extraction (STR and mtDNA).<sup>[73]</sup> However, taping is more precise and is performed by gel-film or single or double-sided sticky tape to lift off the material from the fabric.<sup>[74–77]</sup> An



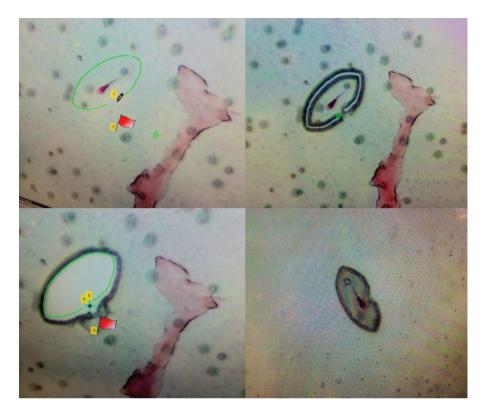
**FIGURE 5.8** Narrowing down the location of sperm on washed or unwashed clothing by use of forensic light source. Note that 'innocent' transfer might have taken place; therefore document every single step of the examination and all locations photographically.

advantage of this method is that the tape may be brought onto a microscopic glass slide where it cannot only be inspected and stained but the cells can also be cut out without danger of jumping off or mixing with DNA from other sources. When it comes to skin on clothing, tape or 'mini-tape lifting' often leads to better results than swabbing.<sup>[78]</sup>

#### SINGLE SPERM AND MICRODISSECTION

Isolation of DNA from single sperm within vaginal cell mixtures is possible by preferential extraction methods (i.e., differential lysis: vaginal cells are digested more easily). If there are less than 250 pg of DNA available, e.g., on few sperm heads on a microscope slide, the use of laser capture microdissection for the isolation of spermatozoa is preferable (Figure 5.9). Such slides are often available in laboratories which perform a quick visual check of fresh swabs from rape and other sexual assault cases. Sperm heads are usually robust and even survive heating on a heat plate to fix them to the slide and staining, e.g., with Kernechtrot (nuclear fast red) and picroindigocarmine ('Christmas tree staining' due to the green and red colors) often used to visualize sperm heads and vaginal cells microscopically.<sup>[79]</sup>

#### Forensic DNA Samples



**FIGURE 5.9** Laser microdissection of sperm head out of the matrix after embedding and 'Christmas tree' staining. The single cell cut out is shot directly into the DNA free reaction tube.

After laser dissection, low copy number PCR may help in obtaining a suitable DNA profile.<sup>[80]</sup>

At wavelengths of 320–400 nm (compared to infrared 812 nm), the dissection is precise and cutting enables single cell and subcellular microdissection. After photovolatilization of the cells, the layer containing the cells is ejected against gravity and either simply falls (by the force of gravity) or is directed by electrostatic forces into the reaction tube. Since the absorption maxima of DNA, RNA (and proteins) lie outside the operating wavelength, no harm to DNA and RNA occurs.<sup>[81–84]</sup>

#### STORAGE AND EXTRACTION

Dried biological samples should be stored in standardized paper bags (envelopes, brown paper bags) in a dry and cool environment (Figure 5.5). This will preserve the DNA over months to years. If dry samples need to be stored for more than 2 years, freezing below  $-20^{\circ}$ C is recommended. To avoid paper layers sticking to each other in the freezer, the envelopes should be put into plastic bags. Never write on plastic

surfaces that become frozen because any type of ink will easily come off. Use paper labels instead.

In temperate parts of the world, DNA was successfully extracted out of clothing and smears on slides that had been stored more than 10 years in dark environments at room temperature. In tropical countries, freezing is always necessary because of the high humidity, which allows bacteria and mold to build up.

Biological stains on glass slides, either embedded (histological tissue samples) or just regular smears (vaginal smears or blood), generally lead to good extraction results. The slides should be stored in standard cases for microscopic slides. Alternatively, they can be fixed with sticky tape inside of a paper envelope. Traces of dust generally do not affect the quality of dry stains but should obviously be avoided.

Insects collected at crime scenes or from corpses should not be dried because museum beetles will frequently destroy the samples within months. The insects should be preserved in 90% EtOH. At room temperature, DNA extraction of such material will then be possible up to several weeks after storage; at temperatures below –20°C, extraction will be successful for several years.<sup>[58,85,86]</sup> Never use formalin to preserve samples; it will degrade the DNA.

Cigarette butts, envelopes with stamps, fingernail clippings, and dried nasal secretions should be stored dry in paper bags, envelopes, or cardboard boxes. Fingernails can be thoroughly swabbed if clipping is not an option.<sup>[87–89]</sup>

Because telogenic hair and broken-off hair shafts have been successfully used for DNA extraction, hair should be carefully stored, e.g., by attaching one end of every hair with sticky tape to the inside of an envelope or between two layers of filter paper. If hair is collected by the police using sticky tape for fiber collection, all material (fibers, lint, and hair) should remain on the tape until extraction becomes necessary.<sup>[3,4]</sup>

If the samples are 'challenged', i.e., either not clean or stick to tape, quick extraction with lysis buffers like BTA<sup>TM</sup> may be used to get access to the DNA by destroying matrices of teeth and bone as well as of adhesive-containing substrates including chewing gum, cigarette butts and tape lifts.<sup>[90,91]</sup>

The success of later DNA typing depends on the number of cells transferred to and from the material used as evidence. Cigarettes, bloodstains, and headwear have high success rates for DNA extraction, even after prolonged dry storage. Cartridge cases, crowbars, and tie-wraps are less successful. If the DNA concentration decreases below 6 pg/ $\mu$ L, only 5% of the extracts provide meaningful DNA profiling data in a standard STR setting. Traces with a concentration above 100 pg/ $\mu$ L generally result in DNA profiles that can be used for DNA database storage.<sup>[92]</sup>

It should be noted that in forensic laboratories with many different types of mostly swabbed stains, simple procedures with few steps may be superior to commercial extraction kits as well as protocols with many manipulations. For example, a successful extraction in a high-throughput yet not automated laboratory in a metropolitan city is a 30-minute incubation of parts of the swab with 0.01% SDS and proteinase K at 56°C, followed by an incubation at 100°C for 10 minutes. Chelex-100<sup>TM</sup> may be added but may pose problems for automated liquid handling systems, cause

loss of DNA, and is therefore not necessary in high-throughput method of stains containing low amounts of DNA. The extracts are then concentrated and SDS removed at the same time by one centrifugation step in Microcon<sup>TM</sup> 100 tubes; 1 ng poly(A) RNA helps to increase the amount of DNA recovered.<sup>[93]</sup>

#### EXTRACTED DNA STORED IN BUFFERS

Depending on the applied extraction method, DNA stored in TE [10 mM Tris–HCl (pH 7.5), 1 mM EDTA] or similar buffers may be stable for weeks (after Chelex extraction) or months (formerly, phenol/chloroform extraction or today, use of spin columns) in the refrigerator at +4°C to +12°C. Freezing of extracted DNA in TE buffer below  $-20^{\circ}$ C will preserve the sample for years. Before freezing, it is strongly recommended to distribute the DNA in small aliquots (e.g., 10 µL each) to avoid repetitive thawing and freezing of single samples. Dry storage is preferred for original stains and swabs.

#### **EMERGENCY BUFFERS**

Under difficult field conditions, a possible standard storage buffer for extracted DNA is TE buffer. Before its use, the buffer is autoclaved or cleaned with a sterile filter. It can then be stored at room temperature. Under extreme conditions, if drying of the samples is impossible (because of dust, humidity, chaotic mass disaster environments), TE can be used to collect samples by aliquoting 1 mL TE into sterile, DNA free 1.5-mL plastic tubes. The collected biological stains can be put inside these emergency containers. DNA must still be extracted as soon as possible, and the samples should be stored as cool as the situation allows.

If more than a few hours are expected to pass before freezing or drying is possible, any solid biological sample in the field should be stored in centrifuge tubes containing aliquots of 95% EtOH. At room temperature, this will preserve the sample's DNA for weeks.<sup>[94]</sup> Recently, 100% isopropyl alcohol or 70% ethanol (v/v) alcohol were successfully used for storage of swabs that were in danger of developing mold.<sup>[95]</sup>

#### MAIN DESTRUCTIVE INFLUENCES ON DNA

Under the influence of UV light (including sunlight) and acids, DNA contained in biological stains as well as extracted DNA breaks into pieces (degrades). Depending on the intensity of fragmentation, PCR is often possible. Humidity does not directly affect DNA but will allow mold and bacteria to destroy the sample including the DNA within days. Frequent freezing and unfreezing of stains or extracted DNA will also lead to degradation. Household use of detergents and cleaners does not necessarily destroy DNA.<sup>[96]</sup>

Sperm heads on fabric may survive machine washing at  $30^{\circ}C-40^{\circ}C$  if no bleach was used. However, for detection of such stains, narrowband, fixed-wavelength lighting is minimally successful at higher washing water temperatures, probably because most of the seminal fluid is dissolved during washing whereas sperm

heads stick to or in between the fibers. In the beginning, acid phosphatase tests with an extended cutoff are still highly sensitive. In still ocean water, spermatozoa on cotton fabric are undetectable after 12 hours, in swimming pool water after one week, yet with no upper limit of detectability for tap or river water, even though a decreasing trend overtime occurs.<sup>[97]</sup> Semen-stained underwear DNA led to recovery of between 13 and 55 ng/µL DNA with successful STR typing in all such cases. When semen-stained underwear is washed after a month at 30°C, some semen stains can still be detected by narrowband forensic light sources or prostate specific antigen, and all stains can be successfully DNA typed.<sup>[98]</sup>

#### CONTAMINATION

Under conditions of normal case work, contamination is only observed after careless manipulation or purposeful spraying of high (nanogram) amounts of DNA near or directly into open tubes before PCR. Secondary transfer via door handles, etc. is only a problem under extremely careless, unprofessional conditions.<sup>[99–101]</sup>

Obviously, mixtures of DNA might be present in the samples themselves. Mixtures of epithelial cells with sperm can be separated by differential lysis (separation of sperm from epithelial cells).<sup>[102]</sup> Other mixtures may show distinctively different peak heights after electrophoretic separation of the PCR products. For example, an object at a crime scene may have been touched by Person A days before a biological stain (such as blood) of Person B was deposited on the same surface. In that case, a DNA mixture might be present later. It can often be detected by the different peak heights of the STR alleles. Mouth-to-mouth-kissing is a lesser practical problem since the other person's DNA inside of the oral cavity of the other person will not show in STR systems already 1 minute after the end of the kiss.<sup>[103]</sup>

Irrespective of the possible presence of mixtures, swabbing is always recommended if the items cannot be moved, are bulky, or if the stain is located on a person. Subsequent procedures like differential lysis should not be performed before DNA extraction becomes necessary. Generally, once evidence examination is completed, all biological samples should simply be stored cool and dry, and left intact as long as possible.

Care must however be taken to avoid contamination and misinterpretation of DNA that was transferred by persons not related to the (criminal) event, e.g., persons present at parties (skin), newspaper or journal readers, clothing stored on the same shelf, etc. (secondary transfer).<sup>[17,104–107]</sup> Tertiary as well as non-transfer is also possible. Accused persons sometimes argue that their garment was used by the 'real' offender who did not leave traces. In sweat bands, three subsequent wearers leave their respective, full profiles on the outside (67%) and on the inside (80%) whereas profiles of only the first wearer are hardly found (one of 200 cases); a single profile of only the second wearer may be apparent in 7% of samples. It is therefore highly unlikely to wear/use a piece of clothing for even a short period of time without leaving DNA behind.<sup>[69]</sup> There are numerous guidelines concerning the handling of DNA evidence but they are sometimes limited by local regulations, education of personnel and agencies involved.<sup>[108–110]</sup> General standardization of evidence examination and procedures in the form of International Organization for Standardization (ISO) guidelines are nearly impossible due to the different composition of stains, laboratories, and evidence examination teams. A DIN/ISO (Deutsches Institut für Normung) attempt to standardize 'recognition, recording, recovering, transport, and storage of material' was retracted.<sup>[111]</sup> Even though laboratory gloves are not a main source for contamination, other surfaces are, including the body and clothing of the person collecting the stain. Continuous training to keep avoiding contamination in the light of today's single cell DNA approaches is necessary.

#### WITHDRAWAL OF SAMPLES OUT OF STORAGE

If parts of a stored biological sample need to be withdrawn for DNA extraction, forceps and scissors must be wiped with paper towels and 70% EtOH (or methylated spirits) every time they are used. In routine use, cross-contamination caused by wiped, smooth-surface forceps has not been observed. An exception is forceps with grooves. They must be autoclaved before every use because the groves quickly fill up with contaminants.

Still, especially during evidence examination and withdrawal, it is essential to take care of cross-contamination caused by contaminated distilled water, touching the swabs with used gloves, etc. Standard bacteriological procedures are an optimal guide.

#### SAMPLE RETAINMENT

It is recommended to always retain at least half of a stain in storage. One reason is that extracted DNA in liquid buffers is less durable than the original, dried stain. In addition, the defense should have a chance to reexamine the stain beginning with the original sample, not the extracted DNA. Only if DNA extraction and PCR seem to fail because of low amounts of DNA, stored samples be used up completely. This needs the consent of the prosecutor's (D.A.'s) office. Even in these cases, at least a minute amount of the original material should be stored so that future DNA technologies may be applied later on.

#### CONCLUSION

Collection of biological stains should be documented by photographs and drawings. Dry and cool storage will allow biological samples to be stored over years.

Extraction of DNA should be performed only if necessary for a current investigation. The original stains should never be extracted completely. Contamination in the laboratory does not occur if the sampling is performed by trained personnel. Because many surfaces and even stains like fingerprints (skin lines), corneocytes on ropes, telogenic hair, the surface of skin after showering, etc., may contain material that is suitable for DNA typing, intelligent criminalistic decisions have to be made before collecting the evidence.

Intense swabbing and the use of sexual assault kits are simple yet very important procedures that guarantee maximum yield of DNA and collection of biological material even if it is not visible at the moment of collection. Even difficult stains such as feces can be extracted and should be stored frozen whenever possible. Under extreme field conditions, 90% EtOH may be used as a collection and storage liquid.

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