Review Article

# The Importance of Biological Traces in Investigative and Criminal Proceedings

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**Abstract**

Biological traces originating from a human, whether it is a victim or an attacker, or from other persons who happened to be there accidentally or intentionally – are of great importance. These are traces of blood in various quantities and forms, traces of semen, sweat, hair, feces, urine, vomit, meconium. The method of collecting, packaging and processing these traces falls within the scope of the work of the police forensic service, and the found material is processed in forensic laboratories. The aim of this paper is to examine and explain the importance of biological traces in investigative and criminal proceedings, i.e. their use to determine the identity of the person to whom they belong, the relationship between the victim and the attacker during the commission of the crime, the type of participation in a traffic accident, etc. Thanks to these traces, it is possible to find answers to the questions sought by investigative authorities related to a specific case.

**Keywords:** biological traces; blood;semen; sweat; hair; feces; urine; forensic medicine.

## Introduction

Forensic autopsy, as an indisputable method of determining the cause of death and numerous facts related to it, is only one part of the investigative actions in the process of collecting data and evidence that will provide the investigative authorities with answers to the questions asked in connection with a specific case. Forensic technical research, in addition to photographing the place of death or related to it, and everything that preceded and followed it, includes collecting traces that were left on the corpse, its clothing and footwear, and the surroundings. Also, traces of struggle, contact between the attacker and the victim are collected, among other things, from the clothing and footwear of the possible attacker, from his body parts, the vehicle in which they were after the event, and from the room in which the event took place, or in which he stayed afterwards.[1,2,3]

### Biological traces of human origin

The investigator is interested in clues that arose during and in connection with the event, therefore, those that are relevant to criminal law, and not others that are unrelated to the event.[2,4,5]

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To eliminate any doubt about the identity of the sample, each trace or trace-bearing object must bear a permanent identification mark from the moment it is fixed at the scene until the procedure is completed.[6]

**A trace becomes material evidence if the elements that determine its origin, or the method of its creation, are identified and proven (identification).**

An expert witness does not always have the objective ability to determine or rule out with absolute certainty, by applying scientific methods, the origin or manner of occurrence of a trace, and therefore to express his opinion in degrees of probability, where the assessment "possible" means about 50% of the absolute value, "low probability" about 60%, "high probability" about 80%, and "very high probability" about 90%. In such cases, the evidence is subject to the assessment of the court.

Material traces, can be of various origins. They can come from weapons and implements used to commit murder or inflict injury, or they can indicate the time of death based on changes in the environment or the place where the body was found. [1,2,7,8,9]

The results of laboratory research on biological traces of human origin are used to determine the identity of the person to whom they belong, the relationship between the victim and the attacker during the commission of the crime, the behavior of the victim and the perpetrator after the event. In addition to traditional research, the most commonly used method today is the determination of DNA profiles.[3,10,11,12]

**Blood**

Blood can be found at the scene of an incident and in its surroundings in many forms, depending on the size and type of injured blood vessel, the amount of blood that has leaked, the relationship between the injured body part and the surface, body movement, etc.[1,2,3]

Blood can also be found on objects used to commit a crime (e.g. a knife).[5,6,7]

If blood drips vertically onto a surface, depending on the height, circular spots with uneven edges in the form of short arms are formed around the spot. If blood drips at an angle to the objects on which it falls, the spots have a more or less elongated shape, depending on the sharpness of the angle. When an injured person moves, the blood trail has a specific shape that indicates the direction of movement of the body or the part of the body from which the injured person is bleeding. This trail is in the form of an elongated blood stain with several smaller elongated spots that are created in the direction of movement.[1, 2, 3, 13]

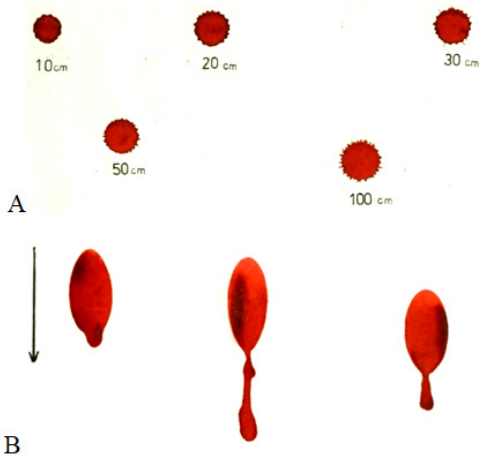


**Fig. 1: A -Traces of vertical blood dripping from different heights; B -Traces and direction of blood dripping down a vertical side (Dobričanin, 2004.)[2]**

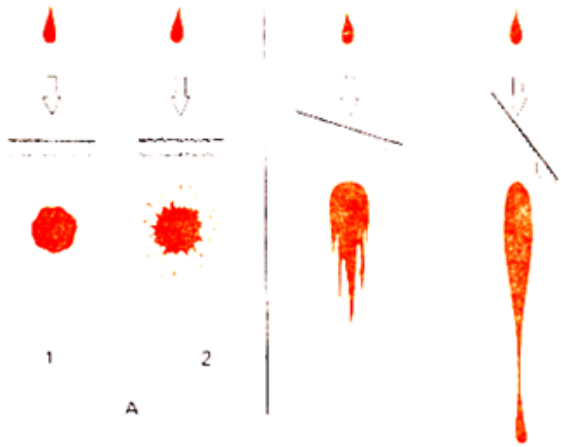


**Fig. 2: Blood dripping onto a horizontal and inclined surface(Dobričanin, 2004.)[2]**

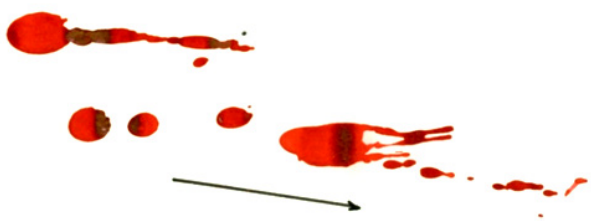

**Fig. 3: Direction of movement of the person from whom the blood traces originate(Dobričanin, 2004.)[2]**

**Fig. 4: When you hit a bloody surface, blood sprays in various directions(Dobričanin, 2004.)[2]**

*Collection and transportation of blood samples*

If blood is on fabric, it is collected with it, and if it is on shoes, glass or smooth surfaces, it is removed with filter paper with water or saline. Blood from walls and floors is removed by scraping, and if it is on wood or paper, it is collected with that material. In the case of traces under the nails, the nails are cut off. The collected traces are placed in plastic or paper containers, properly labeled and transported for further processing.[1,13]

Wet blood stains and clots are collected in a test tube with saline. [5,6,13]

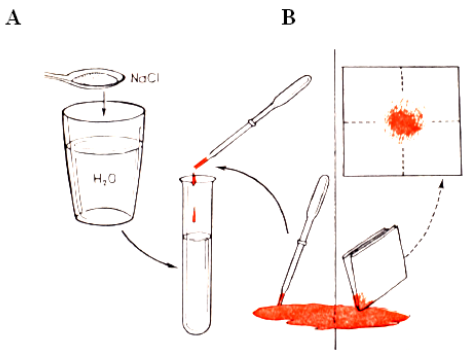


**Fig. 5: Collection of liquid or clotted blood samples from a wet spot: A – into a test tube with saline solution and B – onto a clean cloth or filter paper(Dobričanin, 2004.)[2]**

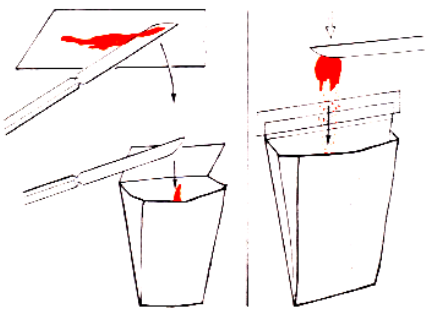

**Fig. 6: Removing dried blood stains from horizontal and vertical surfaces (Dobričanin, 2004.)[2]**

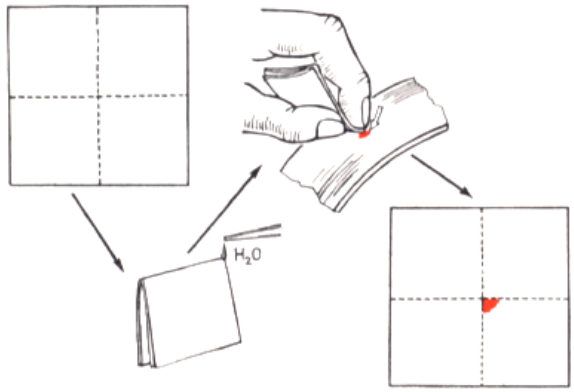


**Fig. 7: Removing small blood stains from a non-absorbent slanted surface (Dobričanin, 2004.)[2]**

*Blood trace analysis*

Various tests are used to confirm whether a stain is blood. Orientation tests react to the presence of catalase and peroxidase in the blood, while selective tests detect specific crystals in the presence of hemoglobin.

Basic tests include:

1. Benzidine test - Shows a blue color change in the presence of blood.
2. Leucomalachite green test - A green color appears if blood is present.
3. Luminol test - Uses chemiluminescence to detect blood.
4. Kastle-Meyer test - A pink color change indicates the presence of blood.

Selective methods include the formation of specific crystals that indicate hemoglobin, such as Tichmann crystals and reaction with acetone and hydrochloric acid.

Once the stain is determined to be blood, methods are used to determine the origin of the blood (human or animal), as well as whether it is male or female, or if it is menstrual or fetal.

Methods such as precipitin reaction and electrophoresis can help determine the origin of the blood, and specific detectors such as Barr bodies for females and fetal hemoglobin tests for newborns.

A recent study shows that attenuated total reflectance Fourier transform infrared (ATR FT-IR) spectroscopy combined with chemometrics can effectively estimate the time since deposition of blood

from a crime scene. In their study, the researchers analyzed 960 bloodstains on a variety of surfaces, both indoor and outdoor, over a period of 212 days to develop models for predicting the time since deposition (TSD). They focused on four different surfaces: white cotton woven fabric, plain cellulose paper, filter paper, and glass.[14]

The research team used ATR FT-IR spectroscopy because it provides a detailed molecular fingerprint, allowing them to track how the composition of the blood changes over time. PLSR models demonstrated strong predictive capabilities, with residual predictive deviation (RPD) values exceeding 3 and $R^2$ values greater than 0.90. Notably, the models performed better on non-rigid surfaces, such as cloth and paper, than on rigid surfaces such as glass. These models can reliably estimate how long a bloodstain has been present, whether it is on cloth in a dark indoor environment or on paper exposed to outdoor conditions. [14]

UV-vis spectroscopy has been extensively studied, but near-infrared (NIR) spectroscopy, despite its potential advantages, has received less attention. A recent study addresses this gap by comparing NIR and UV-vis spectroscopy for forensic dating of bloodstains.15 The study aimed to evaluate NIR spectroscopy for estimating the age of bloodstains. Classical preprocessing methods such as Savitsky-Golay derivatives and standard normal variance (SNV) transformation were used, along with targeted strategies such as cluster centering, which have been shown to be effective through principal component analysis (PCA). Partial least squares (PLS) regression models were then applied to assess the accuracy of the methods in estimating the time since deposition (TSD) of bloodstains. The results showed that both NIR and UV-vis spectroscopy were comparable in estimating TSD, with root mean square errors of prediction (RMSEP) of approximately 40 hours for UV-vis and 55 hours for NIR. These findings suggest that NIR could be a promising tool for dating bloodstains, with further improvements over previous studies. [15]

The authors conclude that NIR spectroscopy, with the right chemometric strategies, holds great promise for forensic dating of bloodstains, but further research is needed to refine these methods for practical use in real cases. [15]

**Sperm**

Traces of semen can be found in cases of rape, murderof women, in places such as the oral cavity, female genitalia, anus, and other parts of the body. Traces can also be found on clothing, underwear, and bedding.

Sperm is whitish-yellow in color and leaves characteristically shaped stains. They can be seen on the skin under ultraviolet light. If the presence of sperm is suspected, swabs or scrapings are taken from the skin, and the suspicious stain is cut from the clothing and bedding.

The origin of the stain is determined by semen analysis, as well as acid phosphatase tests. If sperm are absent, microcrystallographic tests such as the Florence and Barbieri tests, which are not specific for sperm, are used. [2,3,5,6,16]

Acid phosphatase is an enzyme secreted by prostate cells and is a regular component of seminal fluid. The amount of acid phosphatase is expressed in King-Armstrong units. There are about 2,500 King-Armstrong units of acid phosphatase in 1 ml3 of seminal fluid.

"Phosphatabs Acid" is reagent is produced in the form of two tablets: yellow and silver. The reaction itself is carried out as follows: the stain, which is assumed to contain traces of sperm, is placed together with the substrate in a porcelain laboratory cup and two milliliters of distilled water are poured over it. After standing for 30 minutes, the extract is separated and placed in an amount of 0.2 ml in a prepared test tube, and a silver-colored reagent tablet is placed on it and crushed with a glass rod. Depending on the temperature in the laboratory, this solution is left in the test tube for about 20 minutes. After that, a yellow reagent tablet is placed in the test tube, which is also crushed well. After 3 minutes, a brownish-purple color appears, which may vary in intensity. The color intensity is compared with a graph showing the determined values of acid phosphatase for individual degrees of color change. [5,6,16]

**Next-generation sequencing (NGS)** marks a major breakthrough in forensic genomics, enabling high-throughput in-depth genetic analysis and is particularly valuable in cases where sperm are present in low quantities or have undergone degradation.

Benefits of NGS in forensic sperm analysis: High sensitivity[17], Comprehensive profiling[18] and Resistance to degradation: NGS can effectively process degraded DNA samples, which is a common problem in forensic investigations where sperm may have been exposed to adverse environmental conditions.

**Artificial Intelligence (AI)** capabilities in forensic sperm analysis, such as image recognition, pattern identification, and predictive modeling, offer substantial advantages.

Benefits of AI in Forensic sperm analysis:

1. Automation: AI can simplify sperm identification and counting, reducing human errors and speeding up analysis.[19,20]
2. Increased accuracy.[17]
3. Predictive analytics.[21]

**Flow cytometry (FC)** is a powerful method that evaluates the physical and chemical properties of cells or particles as they pass through a light beam in a fluid stream. In forensic semen analysis, this technique allows for more precise identification and quantification of sperm cells.

Benefits of FC in forensic semen analysis:

1. High throughput: FC can process thousands of cells per second, making it ideal for analyzing large volumes of forensic samples.[18]
2. Multi-parameter analysis. [22]
3. Specificity.[20]

Microfluidic devices, or lab-on-a-chip systems, manipulate tiny fluid volumes through channels on a microscopic scale. These devices offer precise control over biological samples, making them ideal for forensic sperm analysis.[23]

These devices enable highly efficient sperm analysis, especially in cases where sample volume is limited.[17,18]

**Secret from the nose**

The forensic significance of this secretion comes down to determining the group in dried stains on pocket handkerchiefs from the scene of the incident in order to "identify" the owner.

In practice, however, these stains do more harm than good: when traces of blood or semen are found on the tissue, the nasal secretion stains significantly interfere with the analysis and make the interpretation of the results more difficult.[4,5,7]

**Sweat**

Sweat interferes with analysis. For example, if a blood type 0 stain on a cloth soaked in sweat from a type A person gives a result that corresponds to type A, the expert must take this influence into account.[1,2,3]

**Urine**

Urine stains are rarely taken for analysis. In practice, this is done when we want to check whether a person has been lying unconscious for a long time or has been forcibly confined or immobilized (tied) in one place and/or in cases where it is necessary to determine whether urine was maliciously poured on a helpless person.[2]

**Excrement**

Feces may be submitted for examination in cases of malicious coating of objects, or even a person, with feces, in cases where the perpetrator relieves himself at the scene of the crime, in cases where the perpetrator steps on feces found at the scene of the crime with his shoes, etc.[5,6,10,11]

The possibilities of identifying the person from whom the feces originated are minimal: if intestinal parasite eggs are found in a disputed stool sample and such eggs are not found in the feces of the suspect, the finding will provide grounds to exclude the suspect.

**Hair**

Hair is a common biological trace that serves to shed light on significant facts related to the commission of a crime.[24,25]

The morphology of hair is such that it cannot be replaced by fibers of any origin.[26] Hair grows an average of 0.5 mm per day. [2,3]

The difference between human and animal hair is based on the ratio of the cortex to the medulla. In humans, the medulla is narrow and the cortex is wide, while in animals the medulla is wide and the cortex is narrow. [2,3]

In the case of a relatively close-range gunshot wound, unburned gunpowder particles can also be found on the hair. In close-range gunshot wounds, the hair is tanned, partially burned. [27,28]

A specially modified absorption method can be used to determine the blood type of people who belong to the secretor group.

The identity of a person is established by determining the DNA profile of the hairs found, which must be at the root, which is compared with the DNA profile of the suspect.

***Hair collection and packaging***

They can be collected with a vacuum cleaner with a filter, tweezers, or clear adhesive tape, or the specific item can be completely packaged.[28]

Individual hairs are packaged in small cellophane bags or smaller envelopes of clean white paper, each of which is marked with its location and placed in a common envelope. It is incorrect to place the hairs directly in a large envelope.[1,2,3]

Due to the serrated structure of the outer membrane, when hair falls out, it easily gets caught on rough objects. [24]

The method of collecting hairs depends on the location of the finding: on smaller objects that can be transported, and on which hairs are found stuck or stuck together by blood, the area with the hairs is tightly wrapped with white paper, and the paper is secured with adhesive tape.[2,3,6,8,24]

Firmly attached hairs, along with objects that can be transported, are best removed with dry fingers, because using tweezers can damage them and, if the grip is not secure, they can be lost. Sometimes it is necessary to resort to using adhesive transparent tape to safely collect hair fragments: a piece of tape with the material taken sticks to another part of the unused tape, which practically makes a ready-made preparation for microscopic examination of the material.

When taking samples from the head of a known person for the purpose of comparison with disputed samples, we must keep in mind the way in which the specific disputed sample was obtained: if it was obtained by plucking, the comparative sample should also be taken by plucking; about twenty hairs are plucked from near the wound on the head.[1,2,9,10]

**Fig. 8: Hair samples from the wound area are taken by plucking, not cutting (Dobričanin, 2004.)[2]**

A new application using artificial intelligence could revolutionize the way scientists study hair. The AI model speeds up and simplifies the process of quantifying hair, allowing a microscope to scan slides and collect images of hundreds of hairs at once. In seconds, it can capture a wealth of high-resolution data that is then processed by a deep learning algorithm that captures the color, shape, width and length of each individual hair.[29]

The researchers trained a computer vision model with artificial intelligence to identify hair using VSU's high-performance computing cluster, Kamiak. With the additional help of an Aperio GT450 microscope at the Washington Animal Disease Diagnostic Laboratory, high-resolution imaging of hair fibers has been automated.[29]

The application has many implications, including forensics. The new technology could not only identify the species from which the hair was derived, but also shed light on the age, health, and ethnicity of people, which could help in criminal investigations.[29]

**Spit**

Traces of saliva are most often found on cigarette butts, postage stamps, and envelopes. In a saliva stain from a secretor, it is possible to determine the presence of amylase and the person's blood group. From a saliva stain, the DNA profile of the person to whom it belongs can be determined.[30]

Regarding the possibility of determining the group in saliva on envelopes and stamps, we must mention the negative impact of glue.[30]

Saliva samples for comparison are taken by having the suspect wet the edge of a clean piece of paper, mark the area with a pencil and let it dry. In

addition, the suspect is instructed to affix the stamp and envelope with his/her saliva, or in cases of cigarette butt searches, to smoke a cigarette with or without a filter, depending on the type of cigarette butt in question.[1,2,3,5,6]

## Conclusion

Traces of human origin are of exceptional importance. A large number of unsolved crimes have been solved thanks to this type of forensic examination, after many years.

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**Ethical clearance:** Not Applicable as it is a systematic review without the involvement of human or animal subjects.

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