

Forensic medicine.

**Part 2. Forensic-medical examination of injuries
caused by action of factors of the external environment.
Forensic-medical examination of the material evidences**

Theme 15. Forensic Medical Examination of the Material Evidences of Biological Origin

Guidelines for students and interns



Судова медицина.

**Розділ 2. Судово-медична експертиза ушкоджень
внаслідок дії факторів зовнішнього середовища.**

Судово-медична експертиза речових доказів

Тема 15. Судово-медична експертиза речових доказів біологічного походження

***Методичні вказівки
для студентів та лікарів інтернів***

МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ'Я УКРАЇНИ
Харківський національний медичний університет

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Затверджено вченою радою ХНМУ.
Протокол № 9 від 17.10.2019.

**Харків
ХНМУ
2019**

Forensic medicine. Part 2. Forensic-medical examination of injuries caused by action of factors of the external environment. Forensic-medical examination of the material evidences. Theme 15. Forensic medical examination of the material evidences of biological origin: guidelines for students and interns / comp. Vasil Olhovsky, Mykola Gubin, Petr Kaplunovsky et al. – Kharkiv : KNMU, 2019. – 12 p.

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Судова медицина. Розділ 2. Судово-медична експертиза ушкоджень внаслідок дії факторів зовнішнього середовища. Судово-медична експертиза речових доказів. Тема 15. Судово-медична експертиза речових доказів біологічного походження: методичні вказівки для студентів та лікарів інтернів / упоряд. В. О. Ольховський, М. В. Губін, П. А. Каплуновський та ін. – Харків : ХНМУ, 2019. – 12 с.

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Substantiation of the Topic. During examination of the place of incident, an expert in forensic medicine, who can be a medical doctor of any other specialty, is obliged to promote the investigator in revealing, fixing and withdrawal of the material evidences of biological origin and provide, when possible, explanations.

Duration of practical classes: 2 academic hours

Purpose of the Practical Class: To study forensic medical methods which are used to determine the material evidences of biological origin, to learn characteristic and peculiarities of forensic-medical examinations of material evidences of biological origin.

Direct purposes of study:

1. To know the theoretical framework of laboratory tests and demonstrate the ability to interpret their results.

2. To be able to describe the material evidence of biological origin at the place of incident.

3. To know how to withdraw material evidence of biological origin from their location.

Basic level of knowledge and skills (before the practical class):

1. Classification of laboratory tests;

2. Principles of blood coagulation;

3. General information on DNA analysis, blood types' identification.

Visual Aids and Material Tools

1. Different laboratory samples and some autopsy reports; additional case materials;

2. Studying tables, photos, and videos.

Technological card of carrying out the practical classes

No	Level	Time, min	Manuals	Place
1	Control of the initial level of knowledge on the topic	10	Oral answering	Classroom
2	Analysis of the blood stains classification	10	Scheme, photos	Classroom
3	Study the forensic medical methods of biological material examination	35	Tables, photos, video	Classroom
4	Description of each forensic medical method's peculiarities	35	Autopsy reports, laboratory samples	Classroom
5	The examination – situational tasks	15	Situational tasks	Classroom
6	Class summarizing	5	–	Classroom

BLOCK OF INFORMATION

Examination of blood

Stains found at the scene of the incident

Blood stains are a source of important forensic evidence regarding:

- Origins of blood stain;
- Distance of blood stain from target;

- Direction from which blood impacted;
- Speed with which blood left its source;
- Position and movement of victim and assailant;
- Number of blows.

One of the important aspects of the visit to the scene of crime is searching for and interpretation of blood stains. Relatively minor blood smearing may also provide significant evidence, such as a smear on the door handle. Heelprints or shoe prints on a bloodstained area of the body help in the identification of the assailant (see the figure below).

The distribution and amount of blood at the scene of the crime may give valuable information about the manner of death, whether it was suicidal or homicidal, and whether the victim struggled or moved about after his/her injuries.

A trail of blood stains will indicate that the victim was wounded at some distance from the place where the body is found. The victim's body and clothing show dried blood streaks running from the wounds towards the feet – it can happen when the victim is attacked while running or in case of suicide. If a victim is injured while he/she was on the floor, the dried blood streaks usually run down along his/her sides.

Blood coming from the arteries of a living person will be scattered in a fine spray over surfaces upon which the person has fallen. Venous bleeding is a slow steady flow producing a pool if the victim is at rest, and separate widely spaced drops, if the victim is walking.

The blood stain size is more dependent on the target surface type than on the falling distance.

The blood spot shape is dependent mostly on the target surface texture. Blood spots that have landed on the smooth glass are consistent and have uniform (circular) shape, while mass blood stains that have landed on a textured surface, for instance a paper or wood, are of different shapes and have irregular edge's features.

Drops that strike solid, non-porous surfaces, resulting in less spatter on periphery, while the spreading drops' surface film remains unaffected. Drops that strike on the irregular porous surfaces cause a bigger spatter of because the spreading droplet is broken by irregularities of surface. Assessment of the fallen distance which is grounded on spot diameter measurements could be mistaken in cases when the texture of the target surface is not taken into account.

The shapes taken by blood spots may be drops, smears, splashes, spurts, trails, and pools (see the figure below).

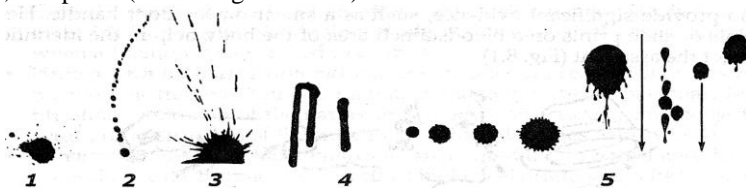


Fig. 1. The shapes taken by blood spots: 1 – drops; 2 – trails; 3 – splashes; 4 – blood streaks; 5 – direction of the fall of blood onto a surface

The direction of the fall of blood onto a surface may be recognized (see figure above). Blood that strikes a smooth surface at 90° results in almost circular blood stain. As the impact angle turns to more oblique the blood spot becomes more lengthened and its width to length ratio rises.

If the height does not exceed a few centimetres, the drop appears as a round spot. If it has travelled 30 cm or more, it shows prickly edges, the projections growing finer and larger in number with the increase in length. When the height is still greater, ray-like splashes break out from the drop, and may be seen up to a distance of 15 to 20 cm.

Splashes of blood striking a surface obliquely may appear like spears or exclamation marks, depending on the velocity and angle of the fall; the pointed end indicates the direction of the motion. If projected onto a wall by an upward sweep of an injured hand, the dots point upwards or if by a downward sweep, downwards. If the long axes of the stains lie horizontally, it is possible to tell whether the drops fell in a forward or backward direction.

Discontinuous spots of blood on the walls of the room indicate splashing of blood from the victim, the assailant, or the weapon, or spurting of blood from a cut artery. It may be possible to see clear drag marks. Smears caused by fingers or palms are helpful in identification. A photograph of blood stains at the scene of a crime is useful.

Preliminary blood test methods

Preliminary methods allow us to establish the possibility that the test spot is blood, not solving this issue in a reliable form. These methods include: ultraviolet research, hydrogen peroxide test, benzidine test and chemiluminescence reaction luminol.

When examined in **ultraviolet light**, blood stains have a dark brown color and a velvety appearance. This method sometimes makes it possible to detect blood stains that have been washed. However, not only blood, but also some other substances, such as rust, have a similar color and appearance in ultraviolet rays.

The **hydrogen peroxide** test is based on the ability of blood to decompose hydrogen peroxide to form water and free oxygen. A drop of 3 % hydrogen peroxide solution is applied to a blood-like spot with a glass rod or pipette. Under the action of catalase contained in the erythrocyte stroma, hydrogen peroxide decomposes with the formation of oxygen bubbles. Hydrogen peroxide is an unstable substance, it quickly decomposes in the light, so it should be stored in a dark glass bottle and, before use, it is advisable to check with a blood stain.

In the **test with benzidine**, a reagent is made consisting of a mechanical powder mixture: barium peroxide (5 parts), basic benzidine (2 parts), citric acid (10 parts). Before use, a small amount of powder (at the tip of the knife) is dissolved in water (1/4 cup). The solution is moistened with a small cotton wool tampon and they are touched to the edge of the trace. In the presence of blood, the tampon becomes bright blue in color.

In a darkened room, if you need to inspect a relatively large area or detect traces of blood after its removal, a reaction with **luminol is used**. A drop of reagent is applied to the edge of the track or sprayed the room. In the presence of blood a bright bluish flash appears – luminescence that lasts for almost a minute.

Evidence-based (reliable) blood test methods

Hemoglobin is an integral part of red blood cells; therefore, the detection of it and its derivatives in stains is evidence of their blood origin. Determination of hemoglobin and its derivatives is carried out in two ways: spectral and chromatographic test.

The spectral test is based on the ability of hemoglobin solutions and its derivatives to absorb light waves of a certain length and to produce streaked absorption spectra. The characteristic properties of the spectrum (the number and location of the absorption bands) are constant and specific for each hemoglobin derivative (hemochromogen, hematoporphyrin).

Chromatographic test. Hemoglobin in blood stains can be detected using chromatography. Chromatographic analysis is a physico-chemical method of separating the components of a mixture of substances when passing them with a current of solvent through a sorbent. After separation, the substance of interest can be developed on the sorbent using color reactions. In particular, hemoglobin is detected using a benzidine reaction.

Regional belonging of blood

If the blood is from the nose, mucus and hair from the nose may be found.

Vomited blood is of chocolate colour and acid in reaction due to the action of gastric juice. In blood due to rape, semen and pubic hair may be found. Blood stains due to boils and sores show a smeared appearance without definite drops of blood, and may contain pus cells and bacteria.

1. Age of blood stains: Fresh stains on light coloured clothes are of bright-red colour that gradually changes to reddish-brown in 24 hours, and brown within a few days, which may become black after a long time. Fresh stains are moist and sticky, and on drying, they stiffen the cloth because of the proteins.

In ordinary conditions, a drop of blood dries in an hour or two. If blood is collected in pools, it may take 12 to 36 hours to dry, depending upon the size and depth of the pool formed. The recently shed arterial blood is bright-red and venous blood dark-red. It can only be stated that the stain is very fresh, recent, some weeks, months, or very old.

2. Source of blood: If the victim and assailant are of different blood groups, it is helpful in establishing the identity. If the stains are on the inner side of the garment, they usually belong to the victim, but if found outside they may belong to the victim or accused.

Collection of blood stains: A clean piece of white filter paper may be used, allowing blood to soak into it, and then drying it at room temperature. A control filter paper should also be sent for examination. Stains on clothing may be scraped off or a fragment of the material cut off.

Serological examination

Serological examination determines whether the blood is derived from human being or from an animal.

Blood types

Blood group could be used in the following cases:

1. To verify if blood stains on clothes, weapons, or in another place are from a particular victim or suspect.
2. To collate fragmentized human remains.
3. In order to resolve inheritance or parentage disputes.

Last time, the DNA profiling is used more than serological blood testing. But, due to its high price and inaccessibility in certain areas, serological tests are still the essential method of forensic examination. The ABO system is used the most and is based on A or/and B antigens expression on erythrocytes and the anti-A or/and anti-B antibodies presence in the serum.

Medico-legal aspects of blood types

The application of blood groupings to medico-legal problems is based on the following principles: A blood group antigen cannot appear in a child, unless present in one or other parents. If an individual is homozygous for a blood group factor, it must appear in the blood of all his children. If a child is homozygous for a blood group factor, the gene for the same must have been inherited by it from each of its parents. The blood group characters are peculiar to the individual and are unchanged throughout life.

Identification by DNA profiling

The DNA is present in sperm, hair bulbs, as well as any tissue having nucleus. The RBC (red blood cell) loses its nucleus early in its life span whereas white blood cells remain nucleated. Just a several hair root bulbs on the instrument of murder, when found in the crime suspect's possession, could be matched with the autopsy blood sample. In the same way, that is possible to compare a semen sample from the vagina of a rape victim with a DNA of suspect's hair or white blood cells. As every cell within an organism has the same DNA, there is no need to compare the DNA from blood with blood or hair with hair only. Samples which are frequently analysed for DNA in forensic cases are: blood stains, liquid blood, saliva, semen, hair bulbs, mouth swabs, cellular tissue.

Polymerase chain reaction (PCR)

Using this technique, it is possible to copy small fragments of DNA in a test-tube resulting in a many million times' increase in the DNA available for testing.

Samples required for DNA profile testing: Live tissue and blood are the best materials, but autopsy samples can be used as long as marked putrefactive changes have not occurred, sufficient to destroy nuclear chromatin. In sexual offences, the most material that can be obtained, such as fluid from the vagina taken by pipette, should be collected. Multiple swabs are a second best, but can still be used. Hairs, pulled out to secure cellular material in the root bulbs, can also be used for data profiling.

Seminal fluid

Forensic medical examination of discharge is carried out at investigation of the criminal cases connected with sexual crimes.

Forensic medical examination of sperm includes:

- Preliminary tests on sperm: visual inspection including survey under the ultraviolet rays. These tests are not obligatory, do not prove presence of sperm on object and are necessary only for selection of the objects which are subjected to further research.

- The proof of presence of sperm in the investigated material;
- Establishment of a specific belonging of sperm;
- Establishment of the group of sperm at isoserologic and immune systems;
- Exception or an establishment of a belonging of sperm to a concrete person (value of DNA methods).

Seminal stains have to be detected in cases of rape or attempted rape, sexual murder of a female, sodomy and bestiality. Fertility of the fluid has to be proved in civil cases, e.g., disputed paternity.

Chemical examination:

Test with the scrape of *potato tubers*: The mechanism of this reaction is based on ability of the potato juice containing ascorbic acid to cause agglutination of erythrocytes of human blood. At presence of sperm, there is a delay of such agglutination, since a reaction between the ascorbic acid of potato juice and the sperm testosterone takes place.

Microcrystalline Florence test: A small piece of fabric about 0.3×0.3 cm in size with a sperm trace is cut out from the investigated site of the objects-carriers and placed on a glass slide, and then 3–4 drops of Florence solution (potassium iodide, iodine, and water) are applied to it. If semen is present, dark brown crystals of choline iodide appear immediately. They are rhombic crystals resembling haemin but are larger, arranged in clusters, rosettes, crosses, etc. Choline originates from the seminal vesicles. The test is not proof of seminal fluid but only of the presence of some vegetable or animal substance. A negative reaction is a proof that the stain is not seminal.

Acid phosphatase test: A high concentration of acid phosphatase is found only in the semen of human beings and monkeys. This is a quantitative test. The amount of acid phosphatase is estimated in a measured specimen, e. g., 1 cm^2 of stained material. It gives a positive reaction in old stains, in the absence of demonstrable sperms, and aspermia. Inhibition of acid phosphatase activity of semen by 1-tartaric acid, though non-specific, is a valuable screening test.

Barberio's test: The reaction depends upon the presence of spermine in semen. A few drops of Barberio's reagent consisting of a saturated aqueous or alcoholic solution of picric acid when added to spermatic fluid produces crystals of spermine picrate, which are needle shaped, rhombic.

The creatine phosphokinase test: Spermatozoa contain a high concentration of creatine phosphokinase, which is more than double than found in any other body fluid. Levels over 400 are almost diagnostic of seminal stains. The basic method is **morphological research** for an element of sperm – spermatozoon.

Electrophoretic methods:

Two methods are in common use: acid phosphatase, and lactate dehydrogenase.

Lactate dehydrogenase: The sperm specific lactate dehydrogenase (LDH) isoenzyme can be separated from other LDH isoenzymes of semen by using polyacrylamide gel electrophoresis. The advantages of this method are: LDH isoenzyme is stable in stains for over four weeks; the isoenzyme pattern of human semen is different from that of commonly encountered animals.

Acid phosphatase: Polyacrylamide gel electrophoresis enables the seminal acid phosphatase to be distinguished from the acid phosphatase present in other substances and even vaginal secretion, on account of differences in mobility. This method is superior to lactate dehydrogenase method since semen can be identified even in the absence of sperms, that is, in azoospermic or vasectomised persons.

Precipitin test: The principle is the same as that for blood.

Group of seminal fluid: The specific agglutinable substances A and B are presented in the semen of secretors. As such, the group of the individual may be determined.

Proof of semen: The only absolute proof of semen is the finding of at least one unbroken spermatozoon.

Urine

Examination of urine and its stains may be necessary in cases of murder and sexual assault.

Urine stains on fabric may appear pale yellow or may have no naked eye appearance of their presence. These give a fluorescence when examined under ultraviolet light. A concentrated extract of the stain may give a characteristic smell due to ammonia evolved by bacterial degradation of urea.

The stains can be identified from the presence of urea, uric acid and creatinine:

Feces

The examination of faecal matter or its stains may be necessary in cases of sodomy and bestiality. Its presence on penile swabs and other garments may be of evidential value.

Saliva

It contains enzymes like ptyalin, glucose-6-phosphate dehydrogenase, various proteins, lipids, chlorides, thiocyanate ions, etc. The stains are identified from the presence of amylase and buccal epithelial cells. Amylase activity can be measured by the starch-iodine test. ABO grouping and species origin can be carried out.

Examination of hair

The examination of hair is of importance in (1) personal identification; (2) identification of hair or fibers found at the Scene, on weapons, clothes, etc.; (3) sexual offences; (4) traffic accidents when specimens of hair are removed from various parts of the motor car, and (5) chronic poisoning by heavy metals.

Issues to be resolved in hair examination:

- 1) the nature of material, i.e. whether it is a hair or it is another material;

- 2) the source of hair – whether it belongs to a human or to an animal;
- 3) the age, race, sex, hair condition, and its characteristics to perform the identification;
- 4) to be used as an evidence in suspected crime.

1. Nature: Human and animal hair consists of the following zones: (1) medulla, (2) cortex, (3) cuticle (see the figure below).

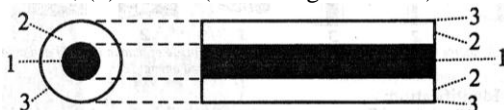


Fig 2. The structure of hair: 1 – medulla; 2 – cortex; 3 – cuticle

2. Source: From the appearance of the cuticle and medulla, the relative size of medulla and cortex, and from the examination of hair in cross-section, an idea may be gained as to the source of hair (see table and figure below).

The differentiating features of human and animal hair

N°	Characteristics	Animal hair	Human hair
1	Texture	Coarse and thick	Fine and thin
2	Medulla	Broad, always present, and continuous	Narrow, may be absent, fragmented, or discontinuous
3	Cortex	Thin	Thick
4	Cuticle	Scales are large, polyhedral and imbricate	Scales are small, flattened, serrated and coronal
5	Medullary index	More than 0.5	Less than 0.3
6	Pigment	Uniform, peripheral, or central	More towards the periphery of cortex
7	Precipitin test with intact root	Specific for animal	Specific for human

3. Identification:

Race: The hair of Europeans is fair, light brown, reddish, or blonde, and of any length; of blacks – short, curly and of Indians, Chinese and Japanese - black, long and thick.

Age: This can sometimes be determined from examination of hair. The lanugo hair of the newborn is fine, soft, downy, non-pigmented, non-medullar, and with smooth edged flattened scales. This is replaced by hair, which is comparatively less fine, pigmented, medullated, and with a more complex scale pattern. Changes in hair due to old age are greying and baldness.

Sex: Sexing of human hair is possible by studying the sex chromatin (X and Y bodies) from hair root cells of the scalp. In addition, beard and moustache hair of the male are the only hair whose sex can be determined. The characteristics and distribution of hair help in determining sex.

Location: Hair from different parts of the human body sometimes present differentiating characteristics, as for example, scalp hair, pubic and axillary's hair, beard and moustache hair, eyelashes, etc.

Special features: Examination of hair is of importance in a variety of circumstances. Although one cannot say that a hair came from a particular individual, by careful comparison, one can often state that it could have come from him. In the comparison of hair debris, grease, etcetera, adherent to the hair, may be of as much value in determining the ownership of hair as the study of its structure. Determination becomes easy if there is some known peculiarity of hair, such as, patchy white hair, dyed hair, bleached hair, and curly or artificially waved hair. Blood group systems can be determined even from a single hair from any part of the body and this may help in identification.

4. Evidence about crime:

Injuries to hair show characteristic changes. In injury, as for example to the head, scalp hair may be damaged by the blow and indications of this may be found in a ruptured appearance of the cortical layer. Hair may get scorched or singed due to burns or firearm injury.

Singed hair is swollen, black, fragile, twisted or curled. The tip of a singed hair swells out to resemble a bulb in shape.

In case of chronic metallic poisoning, the hair retains traces of the poison for a considerable time. Chemical examination in such cases would reveal the presence of the poison in the living as well as in exhumed bodies. The analysis of successive short lengths of hair from the base to the tip gives an approximate indication of arsenic dosage or the intermittent period of such administration. In the last few years, it has been discovered that many drugs such as cocaine, marihuana, are deposited in human hair and they can be detected by special methods.

QUESTIONS FOR STUDENT'S INDEPENDENT WORK

1. Duties of forensic medical expert at the place of incident on revealing blood stains and other objects of biological origin.
2. Methods of biological fluids' analysis.
3. Hair analysis' methods.
4. Withdrawal of the objects of biological origin from the place of incident.
5. Personal identification by analyzing objects of biological origin.

TESTS AND SITUATIONAL TASKS FOR SELF-ASSESSMENT

1. Which method is the best for medico-legal identification of the person?
A. *DNA profiling.* C. *Anthropological.* E. *Biological.*
B. *Osteological.* D. *Examinations of fingerprints.*
2. The one vertical blood streak is revealed on the face of the corpse. It begins from the lower lip and is directed vertically to the center of chin. It is right to consider that bleeding began when the person was:
A. *In vertical position.* D. *In any position.*
B. *In horizontal position.* E. *In sitting position.*
C. *When the head was in vertical position.*

3. Is it possible to estimate the height of blood dropping by the diameter of blood stains on the floor?
- A. No. C. When the diameter on stain was not more than 3 mm.
 B. Yes. D. When the diameter on stain was not more than 5 cm.
 E. When the diameter on stain was more than 10 cm.
4. What is the most illustrative method of the blood presence detection?
- A. *Microcrystalline reaction.* D. *Biochemical revealing of haemoglobin.*
 B. *Microspectral analysis.* E. *Test with benzidine.*
 C. *Test with luminolum.*
5. Choose the most important duty of the forensic medical expert on the crime scene.
- A. *To determine the blood type.*
 B. *To deliver material evidences.*
 C. *To detect material evidences of biological origin.*
 D. *To photography material evidences.*
 E. *To draw up the report of a material evidence withdrawal.*

ANSWERS

1 – A; 2 – C; 3 – B; 4 – A; 5 – C

After the practical class every student should know:

1. Forensic medical methods of biological material examination.
2. Tasks of the doctor at the place of incident on revealing of blood stain, and other objects of biological origin.
3. The blood stains' classification.
4. The blood, hair, semen detection technique.

Should be able to:

1. Perform a blood stain analysis.
2. Evaluate laboratory results on material evidences of biological origin.
3. Withdraw material evidences of biological origin at the place of incident.



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Навчальне видання

**Судова медицина.
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Свідоцтво про внесення суб'єкта видавничої справи до Державного реєстру видавництва, виготовників і розповсюджувачів видавничої продукції серії ДК № 3242 від 18.07.2008 р.