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**Forensic examination of biological material evidence**  
(Forensic Medicine)

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This monograph is intended for students of higher medical schools and masters of the Department of Forensic Medicine and Medical Law. In the monograph, physical evidence of a biological nature, their specific characteristics, importance in solving crimes, finding, collecting, packaging of physical evidence of a biological nature at the place where the incident occurred or where the body was found, to the relevant laboratories information is given on the procedures of sending, the procedural basis of appointment and conduct of forensic examination, as well as methods of inspection. Also, information collected in recent years about new methods of forensic examination of blood and blood stains, as well as forensic cytological and DNA examinations of physical evidence of a biological nature is presented.

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TTA Scientific Council secretary \_\_\_\_\_ Ismailova G.A.

## **INTRODUCTION**

From the first days of the establishment of a legal state and the creation of a democratic society in Uzbekistan, great attention was paid to the protection of citizens' health, life, rights and freedoms by law.

In cases where the health, life and death of a person are at stake in the work of law enforcement agencies, serious crimes, such as murder, defamation, intentional injury, traffic accidents, Forensic examination of physical evidence of a biological nature that serves to identify unknown corpses, victims, accused persons, suspects or contains signs of a committed crime. examination is important. Such objects include parts of the human body (hair, nails, skin, bone remains) and secretions (blood, saliva, sperm, urine, sweat, etc.) and their cellular elements, as well as animal tissues (blood, hair , bones, etc.) serves.

The cooperation of law enforcement officers with experts in the investigation of crimes is of great importance in solving the crime.

Involvement of a forensic expert in the investigation of the scene of the incident, proper finding, collection, packaging of physical evidence of a biological nature, sending it to relevant laboratories in a timely manner, wide use of the possibilities of laboratory tests increases the efficiency of expertise.

Students, including masters in the field of forensic medicine, have a biological nature in order to have the necessary knowledge on the examination of physical evidence, which is considered one of the main branches of the science, and to be able to correctly assess various situations in the examination process. Procedural bases for appointing and conducting expert examinations on physical evidence, procedures for finding, properly collecting, packing, and sending physical evidence to relevant laboratories at the scene of the incident, inspection methods This monograph was prepared.

### **PART I. Physical evidence of biological nature procedural bases of forensic examination**

#### **1.1. Physical evidence of a biological nature and procedures for sending**

## **it to expertise**

According to article 203 of the Criminal Procedure Code of the Republic of Uzbekistan (UzR JPK), the origin, to whom it belongs, whether it was used for certain purposes or suitable for use, whether it passed from one place to another or where it is located Physical evidence is something that has physical symptoms or signs that can be determined to have been changed, affected by one or another substance, thing, process, or event.

Physical evidence includes weapons and other items that, in many cases, may have been used to commit a crime. Physical evidence is different (weapons of the crime, stolen items, documents, blood-like stains left on the items of the crime, hands and clothes of the victim or suspect, saliva, semen, urine , traces of sweat and sweat, hair), not only forensic experts, but also criminologists, accounting experts, technical experts and other experts can be involved for their examination.

In the process of conducting criminal and civil cases, the examination of physical evidence is important for the decision of justice in solving many issues that arise before the operatives of the investigative and prosecutor's office. Therefore, the role of expertise and investigations conducted in forensic laboratory structures in solving crimes against human life, health, and dignity is indisputable. For this purpose, special laboratories were established in the Republican Scientific and Practical Center of Forensic Medical Expertise (RSTEIAM) and its branches in the city of Tashkent, the Republic of Karakalpakstan and the regions.

Physical evidence that forensic experts need to examine can be divided into two groups. The first group is physical evidence that helps to determine the cause of death, the type of coercion and the cause of injuries. For example, a bullet found in the body of a corpse is considered physical evidence, which in turn helps the forensic expert to determine the cause of death and the description of the injury. An electrocution "mark" found on the sole of the corpse's shoe is important in determining the cause of death and the state of the victim at the time of the electrocution. In such cases, the victim's shoes are material evidence.

The second group of material evidence is made up of objects related to

biological origin. Such objects include parts of the human body (hair, nails, skin, bone remains) and secretions (blood, saliva, sperm, urine, sweat, etc.) and their cellular elements, as well as animal tissues (blood, hair, bones, etc.) serves. As a result of the inspection, their nature, characteristics (for example, blood, hair, sperm), and whether they belong to a person or an animal or to which individual is determined.

Examination of physical evidence is of great importance in proving committed crimes. Article 85 of the Criminal Procedure Code of the Republic of Uzbekistan states that evidence consists in collecting, examining and evaluating evidence in order to determine the truth about the circumstances that are important for a legal, justified and fair resolution of the case, in Article 86 of the Criminal Code of the Republic of Uzbekistan and experts and experts are included among the participants in the proof.

Gathering evidence begins with an investigation of the scene of the incident. For this purpose, in order to find traces of a crime, physical evidence, to clarify the circumstances of the incident and other circumstances that are important for the case, the investigator, investigator, or the court may inspect the place of the incident, corpses, animals, surroundings, buildings, objects. and documents must be reviewed.

Inspection of the scene of the incident is carried out if there is information that a crime has been committed in this place or there are traces of it. According to the procedural legislation, any doctor can be involved by investigative or judicial authorities as a specialist to examine the scene of the accident. The purpose of the doctor's participation is to assist investigative agencies in finding and collecting physical evidence of a biological nature. Biological objects (parts of the human body, hair, nails, skin, bone remains, their cellular elements, and secretions - blood, saliva, sperm, urine, sweat, etc.) can be found during the examination, during the forensic examination of the victim and the suspect, and during the examination of their clothes and used criminal weapons.

The doctor should help the investigator in finding such objects, correctly

describing the location of physical evidence, collecting it and sending it to the forensic examination. The doctor, taking into account what happened and what spots are formed after what, can quickly find spots that look like blood or sperm on the bodies in that place without difficulty.

Stains on physical evidence may not always look like stains from biological objects. Sometimes, depending on the specific characteristics of the object with the biological object stain, especially if the traces are on dark, dark-colored materials or objects, metal weapons (for example, knives) are rusted. If the blood stains on the material evidence were deliberately removed (clothes were washed or various fruit juices were applied to the stain, when dyes were dried and in other cases), traces that raised doubts about the presence of blood becomes very difficult to determine. A lot of substances (various fruit juices, paints, etc.) can look like blood stains when they fall or spill on the surface of various objects, or vice versa, sometimes blood stains don't look like blood at all. may not look like blood.

When unidentified corpses, dismembered corpses and skeletal remains are found, the discovery of physical evidence of a biological nature, which is important for crime scene investigation and crime solving, is a complex process. During the examination of the place of suspected dismemberment, it is important to identify the traces of blood and tissues of the corpse on various objects, in particular, on objects that could be used for dismemberment.

Therefore, in such cases, attracting an expert with special knowledge of forensic medicine to the scene of the incident is of great importance in finding physical evidence of a biological nature. The effectiveness of the expertise depends on the comprehensive preparation for the expertise, the correct finding, collection, packaging of physical evidence of a biological nature at the scene of the incident, and sending it to the relevant laboratories in a timely manner. subject to compliance with regulations.

All objects found in the course of inspection or investigative actions, if they are important as material evidence, they will be collected. Before receiving, they are carefully examined and recorded in relevant documents. In the process of

documentation, it is shown where the objects were found and in what condition they are, it is determined whether there are traces of blood stains, what they look like, and where they are located. If the item is retrieved for inspection as an object, the purpose of the retrieval will be indicated. It is advisable to record the condition of the items together with taking photos. Photographs are attached to the collected work materials.

The obtained objects are collected and sealed in an orderly manner by the person conducting the investigation with the participation of impartial people. This, in turn, ensures that they are not misplaced, lost, and that physical evidence is not lost.

In such cases, the process of determining the circumstances of the accident or solving the crime becomes difficult if the physical evidence is collected incorrectly, placed in the wrong packages and not sent to the laboratory for examination in time.

During the inspection of the scene of the incident, objects and traces confirming the occurrence of a crime or related to the details of the work are photographed in compliance with the rules of photography. If necessary, an image of the location of objects relative to each other is drawn. Collected objects and traces are wrapped, sealed, where and when they were collected is indicated on the wrapping, and experts, investigators and impartial people put their signatures.

Physical evidence of a biological nature is the object that is most often examined in forensic examination, blood and bloodstains. It is important not only to be able to find blood at the scene of the accident, but also to correctly describe the size and shape of the traces.

The appearance of bloodstain marks depends on how it was formed. Blood of various sizes and shapes from a large amount of blood oozing from a wound *ponds* appears (Figure 1). If a drop of blood falls on a vertical or inclined surface, it flows downward and *flow* forms (picture 2), blood accumulates more in the lower part of the fistula, and its color is darker. From a height and from a drop of blood falling on a flat surface at different angles *splashes* occurs (Fig. 3).



**Figure 2. Hemorrhage.**

**Figure 3. Blood splatters.**

ar from a drop or splash of blood falling on a horizontal e spot depends on whether the surface on which the blood fell is flat or uneven, what material it is made of, what height and angle it fell on, and the viscosity of the blood. Spots are clearly visible on flat and smooth surfaces, and it is difficult to find them on uneven or bumpy surfaces.

From a drop of blood falling at a right angle from a small height (up to 1 meter), a round or oval blood stain with jagged edges is formed. As the height increases, the jagged edges of the resulting spot become more jagged and light-like lines appear, forming blood splatters around the spot. As the height of the drop of blood increases, the diameter of the formed spot increases, the radius of the blood spatter expands, and the radial lines formed at the edges of the spot become longer. If a drop of blood falls on the surface at an angle, an oval or oblong spot is formed, with a wide, thin part in the direction of the blood. If a drop of blood falls at an acute angle, the resulting stain looks like an exclamation mark, the thin part shows the direction of blood movement. As the height of the drop of blood increases, the oblong wide side and exclamation mark-like traces of the formed stain increase, while the narrow parts become shorter, rays and splashes appear around the stain.

When touching things or clothes with bloody hands, traces of blood are formed, and when holding things with bloody lakes, "bloody traces" remain (Fig. 4). "Images" of footprints, handprints and fingerprints left on objects are of great importance in solving crimes. In addition, the image of the shoes and the crime weapon is also important (Figure 5).



**Figure 5. Bloody images.**

Locations, appearance, color, shape, size, etc. of blood stains help to determine the manner in which the crime was committed, the position of the victim and the criminal in relation to each other, and to determine the active actions of the victim after the injury. .

It can sometimes be difficult to find traces of blood, sometimes washing and wiping them with water or other liquid, painting the floor, and changing wallpaper can be difficult in order to hide the traces of crime. Therefore, you should look for them in inconspicuous, inconspicuous places. These include the inside of the shirt sleeve, the area of the pockets, the area of the lower back of the trousers, the surface of the back of the buttons, the base of the nails, the base of the hair in the bladder area (in rape), the edges and seams of the shoes, the area of the umbrella of hats, under linoleum, plinth includes back surface, floor cracks.

Over time, the composition of the blood on the physical evidence changes, which, in turn, causes the stain to change color. The change in the color of the blood spot is primarily due to the formation of its derivatives as a result of the change of hemoglobin, which is caused by moisture, light, construction, time of formation, exposure to acids and alkalis, and putrefaction of blood. . Blood spots can vary in color from red or dark-red to green. Bloodstain marks can sometimes be "hidden", that is, the same color as the item or fabric on which the blood has fallen. To find such stains, preliminary examination methods using fluorescent light or ultraviolet light are used, because the stains show up well. Hydrogen peroxide ( $H_2O_2$ ) solution and benzidine solution test methods can be used. These tests may also be positive when testing other biological fluids or secretions as presumptive testing methods.

After a blood-like stain is found on physical evidence, it must be taken to a forensic laboratory for examination. If the stains are on small objects (clothes, weapons, or objects), they are removed as a whole. If the blood stains are revived on large objects, the part where the stain is located is cut for examination, and of course a piece is cut from the place without the stain and sent to the laboratory for comparison. If the blood stains are located on things that cannot be removed (wall,

tree), they are scraped from those areas or smeared on wet gauze. The gauze is dried at room temperature. The gauze should not be too wet, because the amount of blood products may decrease in watery conditions. A piece of clean, non-soaked gauze is sent to the laboratory for comparison. The blood stain found in the snow is taken together with the snow and placed in four layers of gauze. Then put the snow in a glass or porcelain container and melt it at room temperature. As a result of snow melting, the blood is absorbed into the gauze, and after the gauze is dried, it is sent to the laboratory. It is not recommended to put the blood in the snow in a closed container, because in such conditions the erythrocytes undergo hemolysis (disintegration) and become unusable for examination.

In order to protect blood-like stains from external influences and pollution, they are covered with paper or gauze, and the edges are sewn or tied to the item. It is forbidden to circle the areas where the stain is located with a pencil, paint or ink, because chemicals get into the bloodstain and make the examination difficult. Each received object is wrapped in a separate clean paper, tied with a string and the ends of the string are sealed by rubbing. The smear and the scrap are put in a clean paper bag, the edges are sewn with a thread, and the ends of the thread are sealed. Then all packages and packages are placed in a plywood box. Physical evidence cannot be sent in soft packaging, as various substances may fall out and make it unsuitable for examination. Physical evidence is stored in the proper order for shipment to the laboratory, which prevents it from being lost, exchanged, and other foreign objects.

In order to determine the group of traces of blood stains found on things, blood samples of the victim and the suspect must be sent to the forensic examination. Blood samples in liquid or dried form are sent together with all collected physical evidence. Blood samples are taken by a forensic expert or a hospital doctor in the presence of an investigator and impartial. A part of the obtained blood (3-5 ml) is poured into a test tube prepared in advance and the mouth is tightly closed. The second part of the blood is poured into a container with gauze and dried at room temperature. Then it is put in a previously prepared

envelope and glued. The investigator seals the envelope and draws up a report on blood collection. The liquid blood sample is sent separately to the laboratory in special containers. This prevents the container from breaking and spilling onto physical evidence during the examination process. Dried blood samples are placed in separate paper bags and sent to the laboratory together with other physical evidence.

Hair-like objects found at the crime scene, preserved in the hands and clothes of the victim or suspect, are taken and placed in separate paper bags, glued and sealed. The packages are marked with where and when they were collected and signed by the investigator, expert and impartial.

When determining the identity of the human hair found in the physical evidence, it is necessary to send the hair fibers found at the scene of the incident and the hair taken from the suspect to the laboratory as a sample for comparison. If the hair sent as physical evidence is scalp hair, then 15-20 hair samples are taken from 5 areas of the suspect's head (forehead, crown, nape and both temples). The hair is cut from the root area of the hair with scissors and placed in separate packages. On the packages, it is written about where the hair is taken from. For example, "forehead hair of citizen Kadyrov K." All bundles are then put into one common bundle and labeled with who the hair belongs to, who collected it, and the date. The edges of the package are sewn with thread, the ends of the thread are glued to the surface of the package, the investigator signs and seals it.

Semen, saliva, sweat, urine, breast milk stains are more difficult to find than blood stains, because they do not have a specific color. Along with a thorough inspection of things, it is advisable to check with the help of a magnifying glass and ultraviolet rays. Discharges mainly give various shades and clarity of blue color, in some cases they can lose their luminescence properties due to changes in the composition of biological objects. It is very important to take a smear from the vagina of the victim in cases related to touching the honor. The smear is removed with a gauze swab and dried at room temperature. It is not recommended to smear the vial, because later it will not be possible to determine the group of sperm due to

its small amount. Collecting, staining, etc. of physical evidence with blood stains is the same as sending blood to the laboratory.

## **1.2. Procedural principles of examination of physical evidence of a biological nature**

According to Article 172 of the Criminal Code of the Republic of Uzbekistan, the appointment of an expert when information about cases relevant to work can be obtained through a special examination conducted by a person with knowledge in the field of science, technology, art or profession shown. The questions put to the expert and his conclusion cannot go beyond the scope of the expert's special knowledge.

Article 175 of the Criminal Code of the Republic of Uzbekistan states that physical evidence and samples taken for expert examination, other material objects whose evidentiary value can be determined by expert examination, the body and mental state of a living person, a corpse, and documents can be included in the objects examined by an expert.

Objects to be examined by an expert, if their size and characteristics allow, should be given to the expert wrapped and sealed. During the inspection, the material object of expertise can be damaged and used to the extent necessary for the inspection. If, after the expert examination, the objects of inspection are left over without being fully spent, they must be returned to the investigator, investigator or court that appointed the expert. Expertise objects are stored in expert institutions, inquiry, preliminary investigation, prosecutor's office and courts in accordance with the rules for storage of physical evidence.

Article 208 of the Criminal Code of the Republic of Uzbekistan provides for the loss, damage of physical evidence during the storage of physical evidence, as well as when sending it for examination or in connection with the transfer of a criminal case to other investigation, preliminary investigation bodies, prosecutor or court, it is indicated that measures should be taken to prevent their violation, merging or mixing.

Article 180 of the Criminal Code of the Republic of Uzbekistan stipulates

the decision and court ruling on the appointment of an investigator, investigative expertise. In the decision or ruling on the appointment of expertise, the reasons for the appointment of expertise, physical evidence and other objects sent for expertise, when, where and in what condition they were found and received, questions to be put before the expert, the name of the expertise institution or the last name of the person entrusted with conducting the examination should be indicated. The decision or ruling on conducting an expert examination is binding for the persons affected by it.

The following documents must be submitted to the forensic laboratory along with physical evidence by the investigator, investigator or court:

- the decision of the investigation agencies on the appointment of the forensic examination of physical evidence or the court ruling;
- a certified copy of the report on inspection and collection of physical evidence;
- a certified copy of the report on taking samples for comparison;
- a certified copy of the primary expert's conclusion and the expert's workbook in re-examinations;
- if the corpse has been examined or the victim has undergone a forensic medical examination, then an expert opinion will be submitted.

When appointing a forensic medical expertise on physical evidence of a biological nature, the investigator, investigator or court has to carry out sampling activities for expert examination for the purpose of comparison. The procedures for taking samples for expert examination are specified in the relevant articles of the Code of Criminal Procedure of the Republic of Uzbekistan.

Article 188 of the Criminal Procedure Code of the Republic of Uzbekistan lists the types of samples taken for expert examination and the methods of obtaining them. If it is necessary to solve the questions, it is shown that they are correct.

Reflecting the characteristics of a living person: biological - blood, hair, saliva, substances released from the human body; psychophysical - dashat;

anatomical - the lines of the skin pattern, tooth molds; Also, samples reflecting voice characteristics and professional qualifications can be taken.

According to Article 189 of the Criminal Code of the Republic of Uzbekistan, if the expert examination is not related to undressing the person from whom samples are taken and does not require special professional skills, the investigator, investigator or the court itself, and if necessary, a doctor, other specialist, expert it is indicated that he has the right to take samples for expert examination.

If taking samples for expert examination requires nakedness or requires special professional skills, a doctor or other medical specialist will take samples for examination on the order of the investigator, investigator or court.

Article 190 of the Criminal Code of the Republic of Uzbekistan states that the persons who can be sampled include the suspect, the accused, the defendant, the victim, as well as the persons who are being investigated for the use of coercive medical measures.

If there are sufficient grounds that traces may have been left by other persons at the scene of the incident or on physical evidence, samples may be taken from these persons for expert examination.

According to Article 191 of the Criminal Code of the Republic of Uzbekistan, the investigator and investigator make a decision on sampling, and the court issues a ruling. The person or body to be sampled in it; person to be sampled; exactly what kind of sample and how much should be taken; when and to whom it is necessary for the person to be sampled to appear; it should be indicated when and to whom the obtained sample should be presented.

Article 192 of the Code of Criminal Procedure of the Republic of Uzbekistan specifies the limit of coercion in taking samples, and it states that suspects, defendants, defendants, and victims who refuse to come for a sample can be forcibly brought. In this case, if the methods used are painless and safe for human life and health, samples are taken by force.

Samples from other persons may be taken by coercion only in the cases

provided for in Article 190 of this Code and in cases related to the detection of venereal disease and other infectious diseases.

Article 193 of the Criminal Procedure Code of the Republic of Uzbekistan specifies the procedure for taking samples by the inquirer, investigator or court, in which the inquirer or investigator calls the person from whom the sample is to be taken, or goes to his place and decides to take the sample, or receives a receipt introducing the decision of the court, and their rights and obligations are explained to this person, expert, impartial, if someone is rejected, this solves the problem.

The investigator or investigator then takes the necessary actions and obtains samples for expert examination. It is possible to use scientific and technical tools that do not cause pain and are not dangerous for human life and health.

It is carried out by taking samples from the corpse, as well as raw materials, products, and other materials, exhumation, cremation, or search.

The obtained samples are wrapped and sealed. Then the inquirer or investigator sends them along with the sampling report to the appropriate expert.

If the sampling was carried out according to the decision of the court, the investigator or investigator who fulfilled this decision will send the samples to the court along with the report on their collection. After the court examines the samples with the participation of the parties and makes sure that they are authentic and fully preserved, the obtained sample is sent to the appropriate expert along with the decision and report.

Article 194 of the Criminal Code of the Republic of Uzbekistan specifies the procedure for taking samples by a doctor or other specialist. The investigator, the investigator or the court sends the relevant person, as well as the decision or ruling on taking a sample from him, to the doctor or other specialist. The question of rejection of a doctor or other specialist, impartial is decided by the inquiry officer, investigator or court that issued a decision or ruling.

A doctor or other specialist performs the necessary actions and takes samples for expert examination. Scientific and technical means that do not cause pain and are not dangerous for human life and health can be used for this. The

samples are wrapped and sealed and sent to the investigator, investigator or court.

Biological evidence found in physical evidence in cases of murder, infanticide, defamation, forced sexual satisfaction, rape, sexual crimes such as forcing a woman to have sex, injury, traffic accidents hair, blood, saliva, sperm and other it is necessary to take samples from discharges. It is very important in proving the crime committed.

In the examination of civil cases, for example, in the determination of disputed paternity and motherhood, in cases of child replacement, blood and saliva are taken as samples from the father, mother and child undergoing examination at the same time.

The procedure for obtaining samples for testing by an expert is specified in Article 195 of the Criminal Code of the Republic of Uzbekistan. After the examination, the expert will attach the samples to his conclusion in a sealed manner.

The investigator or investigator, the court and the parties at the trial examine the samples of the experience presented by the expert, after which they are added to the criminal case as physical evidence.

The methods and scientific and technical means used in taking samples for expert examination must be safe for human life and health. The use of complex medical measures and methods that cause severe pain must be carried out with the consent of the person to be sampled, provided that he is under sixteen years of age or, if he is mentally ill, with the consent of his legal representative, guardians or sponsors. possible

If the sampling is related to the undressing of the person taking the sample, the doctor or other specialist, impartial should be of the same gender as the person taking the sample.

### **1.3. Procedure for conducting forensic medical examination of physical evidence of biological nature**

Forensic examination of physical evidence of a biological nature, in accordance with the current procedural regulations, based on the Order of the

Ministry of Health of the Republic of Uzbekistan No. 227 of July 25, 2011 "Forensic medical examinations in forensic institutions "On the procedure of transfer" of the Ministry of Health No. 152 dated June 1, 2012 Appendix 4 to the order "Rules for conducting forensic biological expertise and investigations" and "Approval of standards for conducting forensic examinations" of the Ministry of Health on April 4, 2015 based on the order No. 82 on

Also, in 2013, "Instructions for performing operations" were developed based on the strict and complete conduct of each type of expertise in accordance with technological processes. As a result, the initially developed methodological manuals and recommendations were updated, new approved technological methods were put into practice, and the sequence of actions, quality criteria and accuracy of results were clearly defined. The achievements and experiences in the field of forensic medicine in the Republic of Uzbekistan and developed countries were taken into account in the development of "Guidelines for the performance of actions". At this point, the period of review for making timely changes to the guidelines for the implementation of actions in each forensic examination institution is set at 3 years due to the fact that the possibilities of independent implementation of research in a specific form or other form are taken into account.

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Forensic forensic examinations of physical evidence in regulatory documents Examination of physical evidence organized in the appropriate manner in the Republican Scientific and Practical Center of Forensic Medical Expertise under the Ministry of Health of the Republic of Uzbekistan and its branches in the city of Tashkent, the Republic of Karakalpakstan and each region the execution is indicated in sections.

In the Republican Scientific-Practical Center of Forensic Expertise, forensic-medical expertises are carried out as re-examinations in individual cases, after conducting initial expertises in the branches of the city of Tashkent, the Republic of Karakalpakstan and each region.

Expertise of physical evidence and biological objects consists of the

following:

- medical forensic examination;
- forensic biological expertise;
- forensic histological examination;
- forensic chemical expertise;
- forensic cytological examination;
- chemical-toxicological expertise;
- biochemical expertise;
- genetic expertise;
- spectrographic expertise.

The Law of the Republic of Uzbekistan "On Forensic Expertise" adopted on June 1, 2010 of the law In Article 11, the post of state forensic expert is held by a state forensic expert who has a higher education, and in special cases, a secondary specialized, professional education, who has undergone further training in a specific forensic expert specialty and in the prescribed manner. it is noted that a citizen of the Republic of Uzbekistan who has been certified as

Expertise of physical evidence is conducted by a medical forensic expert who has undergone special theoretical and practical training in the field of forensic medicine and forensic examination of physical evidence and has the appropriate certificate.

Physical evidence of a biological nature is conducted in forensic-biological departments of forensic medical expertise. Forensic biological examinations are carried out by the person conducting the investigation: an investigator, prosecutor, on the basis of a court decision or a court ruling.

Forensic-biological examination of blood, gauze swabs and other objects taken from the contents of the genital organs is carried out based on the referral of the forensic expert.

The objects of expertise and examinations are parts of the human body (hair, nails, skin, bones, soft tissues) and secretions (blood, saliva, sperm, urine, sweat, etc.) and their cellular elements, as well as animal tissues (blood, hair, bones, etc.)

serve.

The existence of biological objects, their species and group affiliation, morphological and comparative examination of hair, as well as resolving issues in disputed paternity, maternity cases, and cases where children have been exchanged are the goals of expertise and investigations. As a result of the inspection, their nature, characteristics (for example, blood, hair, sperm), and whether they belong to a person or an animal or to which individual is determined.

Before conducting the expert examination, the investigator or the head of the forensic examination institution assigned to him shall explain the rights and obligations of the expert specified in the relevant articles of the criminal and procedural codes, and in the cases of refusing to give a conclusion or knowingly giving a false conclusion, the relevant provisions of the criminal code receives a receipt from him, warning about the responsibility for the articles.

If the forensic expert is a victim, a civil claimant or a defendant, a witness; if he is related to the accused, victim, civil claimant, respondent or their representatives; if he is in a service or other relationship; if there are other circumstances that can be the basis for considering him personally, directly or indirectly interested, he does not have the right to conduct expertise, participate in primary and other investigative activities.

In such cases, it is necessary to give a rebuttal to the expert or for him to give a rebuttal to himself. The question of the expert's refusal is decided by the investigator, investigator, prosecutor or court conducting the case.

During the examination, the forensic expert has the right to ask the person who appointed the examination to clarify and explain the questions that need to be resolved. The forensic expert should refuse to answer questions that are outside the scope of his knowledge or that are not at the disposal of the forensic expert. When determining the circumstances relevant to the case, even if the relevant questions were not asked, the forensic expert may indicate them in his conclusion.

Forensic examination of physical evidence is carried out in separate, spacious, well-heated, ventilated and naturally lit rooms. After the end of working

hours, shelves (cabinets) with material evidence, all rooms of the department of forensic examination of material evidence are locked and sealed with the seal of this department.

Natural daylight is used during inspections. If the inspections are carried out under artificial lighting conditions, the expert's opinion of this event will be explained in the appropriate place.

The following documents must be submitted to the forensic laboratory along with physical evidence by the investigator, investigator or court:

- the decision of the investigative agencies on the appointment of a forensic medical examination of physical evidence or a court ruling (it should reflect the conditions of the incident, the number of items to be sent for examination and the questions to be resolved by the examination);

- a certified copy of the report on inspection and collection of physical evidence;

- a certified copy of the report on taking samples for comparison;

- a certified copy of the primary expert's opinion and the expert's workbook in re-examinations.

If items required by the laboratory have not been sent, the forensic laboratory must request them and may suspend testing until they are received (unless the items are perishable).

In the Department of Forensic Biology, material evidence is accepted by the head of the department only if the documents contain the appropriate official opinion of the head of the branch of forensic medical examination. According to the documents provided, the expert will learn the details of the incident, the questions that need to be resolved, the names of the physical evidence, what traces were found and other things.

During the acceptance of physical evidence, the forensic expert of the department carefully examines the package in which it is located and determines the nature of the package, the condition and integrity of the signatures, stamps and seals on it. The expert examines the box with physical evidence in the presence of

impartial people. In case of misplaced material evidence, unsealed or broken packaging, a document is drawn up in two copies and signed by three employees of the department, one copy of which is sent to the institution that sent the objects, and the other remains in the department. As long as the packages have their integrity, stamps, and seals preserved, they are carefully opened and compared with the list of physical evidence recorded in the decision on the appointment of expertise or other documents.

In cases where some physical evidence is missing or physical evidence not specified in the documents is sent, a document is drawn up in duplicate signed by three employees of the department. One copy of the document is quickly sent to the institution that sent the physical evidence, the other remains in the department. After the document is drawn up, the examination of physical evidence begins.

Documents sent with physical evidence are stored in a safe or a lockable shelf (table). After the end of working hours, they are sealed with the seal of the department. Physical evidence is stored in a separate lockable and sealed shelf (cabinet). Volatile objects sent as physical evidence or samples sent for comparison (for example, liquid blood, saliva, etc.) are tested on the same day they are brought to the forensic laboratory. Perishable items are stored in a sealed container in the refrigerator.

When performing the examination, it is necessary to observe the economical use of objects, because they should be enough for all the necessary types of examination, and a part should remain for the next possible re-examination. In cases where it is not possible to solve the questions before the expertise before the facilities are completely used, the investigative and judicial agencies appointed by the expertise should be notified.

Sheets of physical evidence and documents submitted to the department are numbered, bound and sealed with the seal of the branch of forensic medicine. After the completion of the inspection, the expert will write down all the entered work in the work book and answer the questions.

If a question arises during the forensic examination of material evidence that

does not fall within the scope of the forensic expert's expertise, experts in this field (anatomist, zoologist, histologist, etc.) are consulted. The information and conclusions given by them are used in the expert opinion, and the written answer of the consultant, which remains in the institution of forensic examination, is given as evidence.

After the completion of the expert examination, the physical evidence and samples (non-perishable ones) are sealed (with a complete description), wrapped, and returned to the institution that appointed the expert through the person who has the right to receive them, or by mail, together with the expert opinion and working materials.

After the examination of the liquid blood brought to determine the group and type (before returning to the investigative offices), it is distributed in a thin layer in a Petri dish on a checked gauze at home temperature and dried in the form of a spot in a place that does not get sunlight. Dried blood spots are sealed in separate clean paper bags and returned to the institution appointed by the expert. The blood taken to determine the parentage and parentage of the child is not stored after the examination is completed.

#### **1.4. Expert opinion and its legal evaluation**

According to Article 184 of the Criminal Code of the Republic of Uzbekistan, after conducting the necessary investigations, the expert draws up a written opinion on his own behalf and signs it.

The results of various forensic medical examinations, including forensic biological examinations, are formalized in a document called "Expert's conclusion" according to the criminal-procedural and civil codes. It consists of the following parts: an introductory part containing a brief description of the job description, a review part and a conclusion part.

The following should be indicated in the introduction: the time and place of the examination; conditions during the examination that are important for expert examination (lighting, air temperature, etc.); a procedural document (decision or ruling) that is the basis for an examination; by whom and when the expertise was

appointed; the expert's surname, first name and patronymic, education, specialty, seniority in this specialty, academic degree and academic title, position held; the name and number of the criminal or civil case, the number of volumes, worksheets, the object brought to the examination, the list of samples in the examination of physical evidence; that the rights and obligations of the expert are explained; the fact that the expert has been warned about the criminal responsibility for refusing to give an opinion and knowingly giving a false opinion; who was present during the examination; questions put before the expertise to be resolved; a brief description of the job description.

The details of the case include investigative materials, medical documents, corpses, information from the expertise of the victim, the accused and other persons, necessary for conducting an expert examination and drawing up conclusions.

The inspection section should include a complete description of the inspection process and the information identified in it. In it, which materials of the work were used by the expert, which physical evidence, samples and other objects were examined; in particular, what checks were carried out, what methods were used and their level of reliability are indicated. It includes a complete description of the physical evidence and the traces it contains, the methods and tests used, the reagents, apparatus and equipment used, the description of the results of each type of test with the analysis process, and the examination of the samples submitted to the expert. should take.

The introductory part, case details and examination part together form the protocol of the expert opinion, which is signed by the forensic expert and the persons indicated in the introductory part.

An expert's conclusion is a scientifically based expert's opinion based on the results of the conducted expertise. Stops are made in accordance with the questions to be solved by the expert on the basis of the objective information obtained during the examination. According to the opinion of the expert, they should include the expert assessment of the objective information that is determined during the

examination process and is important for the work, and should be clearly and concretely stated without using medical terms as much as possible. Expert opinion on each question must be supported by factual information.

The expert's report may contain the circumstances, the causes of the crime and the conditions that allowed it to be committed, as well as the organizational and technical recommendations for its elimination, which are important for the case and the expert determined on his own initiative.

The introductory part of the expert's opinion, the details of the work and the verification part are drawn up directly during the examination. The suspension part will be drawn up no more than three days after receiving the results of all expert inspections. The period of conducting expert examinations is determined by their type, nature and volume of expert examinations, and they must be conducted within a period of no more than one month after receiving all necessary materials. In case of extension of this period, the head of the office and forensic medical examination institution where the examination was appointed must be notified in writing about the reason for this.

The expert's opinion is drawn up in at least two copies, one of which is sent to the office that appointed the expert, and the other remains in the forensic examination institution. Expertise of material evidence is formalized with an entry made in the work log during the expert examination process, and based on it, an expert opinion is drawn up after the completion of the expert examinations.

It is forbidden to replace the expert's opinion with various short references and extracts, and to use unapproved forms and questionnaire-type forms in the preparation of forensic documents.

Physical evidence, samples, other objects, as well as photographs, schemes, drawings, left over from the inspection must be attached to the expert opinion.

If it becomes known during the investigation that the materials given to the expert are insufficient or the expert's special knowledge is insufficient, the expert's conclusion should contain the grounds for refusing to answer some questions.

According to Article 185 of the Criminal Code of the Republic of

Uzbekistan, the impossibility of giving a conclusion indicates the absence of factors that allow conducting an examination and the fact that the material and objects available to the expert are not complete for drawing a conclusion. In addition, although the expert has certain special knowledge, but does not provide an opportunity to get an answer to the question put before him, or if the methods and research methods available in science do not allow to give a definite result to get an answer to this question, then the expert will draw up a substantiated document, in which he describes the reasons why he cannot answer the questions put before this expert, and submit this document to the head of the expertise institution and the investigator, investigator who appointed the expertise or send to court. After receiving an expert report on the impossibility of giving a conclusion, the investigator, inquirer may try to solve the questions put before the expert through non-expert investigative actions.

As stated in Article 187 of the Criminal Procedure Code of the Republic of Uzbekistan, the expert's opinion is evaluated from the point of view of its scientific basis and compliance with all the procedural rules established for the examination, together with other evidence collected by the investigator, investigator or the court. If several expert opinions were conducted on a criminal case and the experts did not agree, the investigator, the investigator or the court can decide whether to agree with the conclusions of some experts and not to agree with the conclusions of other experts. must be justified.

The expert's opinion does not have an inevitable evidential force for the investigator, the investigator or the court. The conclusion of the forensic examination can be accepted or rejected by the court or investigation. In the last case, the court or investigative agencies must justify their refusal to consider the opinions of experts in writing, giving concrete reasons. Such a decision of the court or investigative agencies may be the basis for re-examination.

According to Article 176 of the Criminal Procedure Code of the Republic of Uzbekistan, when a re-examination is appointed, the expert may be asked the question of the scientific basis of previously used examination methods. The

reasons for disagreeing with the conclusion of the first examination must be indicated in the decision or ruling on re-examination. The re-examination is entrusted to another expert (commission of experts). The expert who conducted the first examination may be present at the re-examination and give explanations, but he will not participate in the expert examination and conclusion.

## **Control questions**

1. What is meant by physical evidence?
2. What is physical evidence of a biological nature?
3. What are the duties of a forensic pathologist at the site where Moore was found?
4. The rights and obligations of the expert are listed in which article of the JPK of the Republic of Uzbekistan done?
5. Inspection of the place found in Moore is mentioned in which article of the JPK of the Republic of Uzbekistan?
6. What are the procedural normative documents regulating the forensic examination of physical evidence of a biological nature?
7. What can blood and its traces look like at the scene?
8. How are blood samples taken for laboratory examination?
9. What issues are resolved in the examination of blood and its stains?
10. What methods can be used to determine the presence of blood in a stain found at the scene of the accident?
11. What documents should be submitted to the forensic laboratory by the investigator along with physical evidence?
12. In what document are the results of forensic biological examinations formalized?
13. What are physical evidence and biological object expertise?
14. Inspection of the scene of the accident is mentioned in which article of the Criminal Code of the Republic of Uzbekistan?
15. In which article of the JPK of the Republic of Uzbekistan are the objects of expertise specified?

## **PART II. Forensic examination of blood and bloodstain**

### **2.1. Biological significance of blood**

In the practice of forensic medicine, the most frequently examined object of physical evidence is blood. Forensic biological examination of blood in relation to other physical evidence of a biological nature is 80 percent. In order to conduct a forensic examination of blood and blood stains, an expert must first have a complete knowledge of blood.

The science of blood *hematology* (Greek, *hemos* - blood, Latin *logos* - science). This field is wide and deep and studies the phylogeny, ontogeny, morphology, functions of blood, chemical, physical, coagulological, immunological and genetic properties of blood.

***Functions of blood.*** Blood, lymph and tissue fluid form the internal environment of the body, washing all cells and tissues in the human body. Blood performs a number of functions necessary for human life. The first of them *transport* its function is to deliver nutrients to tissues in the body: glucose, amino acids, polypeptides, fats, vitamins, minerals and water, as well as oxygen to the blood in the lungs, and to the kidneys, sweat glands, o It removes the final products of metabolism: ammonia, urea, uric acid and other waste products, including carbon dioxide, from the tissues. Also, the relative constancy of the amount of water and electrolytes in blood cells and tissues (*homeostasis*) keeps; blood transports hormones and other physiologically active substances from the cells that produced them to other cells in the process of chemical interaction in the body - *humoral regulation*; the presence of leukocytes capable of phagocytosis in the blood and immune bodies (antibodies) that neutralize microorganisms and their poisons and destroy foreign proteins constitute the protective function of the blood, and this *of immunity* serves as the most important factor; the energy generated in the process of metabolism in the body is necessary for human life *thermoregulation* provides the process.

***Blood composition.*** The liquid part of blood is plasma (serum) and its suspended elements: erythrocytes (red blood cells), leukocytes (white blood cells)

and blood platelets. Plasma makes up 50-60% of blood volume, shaped elements make up 40-45%, and this *hematocrit index* is called

The total amount of blood in the human body is normally 6-7.5% of the body weight. If the viscosity of water is equal to one, that of plasma is 1.7-2.2, and that of blood is about 5.0. Blood viscosity depends on the presence of proteins and erythrocytes in it. Viscosity can increase when the blood clots, that is, for example, when a person has diarrhea or sweats a lot, that is, when he loses water, as well as when the number of erythrocytes in the blood increases. The specific gravity of whole blood is 1.050-1.060, that of erythrocytes is 1.090, and that of plasma is 1.025-1.034.

**Erythrocyt** - is a red mine cell, which ensures the body's breathing process due to the hemoglobin inside it. 1 mm of a healthy woman<sup>3</sup> there should be 3,800,000 - 4,800,000 erythrocytes in the blood, 4,000,000 - 5,000,000 in a male person. The size of a normal erythrocyte is 6-9 microns, and its diameter is 7.2 microns. An erythrocyte is a microscopic cell of round or oval shape. The osmotic resistance index of a normal erythrocyte is 0.4%-0.99%. An erythrocyte lives for 120 days.

Rupture of the erythrocyte shell, the release of hemoglobin into the blood plasma, coloring it red, and the plasma turning into clear ("lacquered") blood *hemolysis* is called The erythrocyte stroma that is broken and deprived of hemoglobin is called "erythrocyte shadow". For a number of reasons, erythrocytes can break down both inside and outside the body.

Currently, it has been found that there are many antigens on the surface of erythrocytes. The most important of them are ABO, rhesus, Lewis, Lutheran, Kell, Kellano, Duffy, Kidd, Joy, Reith antigens and others.

In 1901, K. Landshtainer and in 1907 Ya. Yansky identified two agglutination factors in human blood erythrocytes - agglutinogen A and agglutinogen B, and two agglutinating substances - agglutinin a and agglutinin b in plasma. In human blood, agglutinogen A and agglutinin a, and agglutinogen B and agglutinin b are never found together, so the body's own erythrocytes are not

agglutinated.

Depending on the presence of agglutinogens in erythrocytes and agglutinins in plasma, the blood of all people can be divided into four groups. According to Yansky's classification, there are no agglutinogens in the erythrocytes of people of group I, and a and b agglutinins are present in their plasma; Group II people have agglutinogen A in their erythrocytes and agglutinin b in their plasma; Group III people have agglutinogen B in their erythrocytes and agglutinin a in their plasma; Group IV people are characterized by the presence of agglutinogens A and B in their erythrocytes and the absence of agglutinins in their plasma. Agglutination is indicated by a plus (+) sign, and its absence is indicated by a minus (-) sign.

Based on the above, all people are classified according to whether they have agglutinogens (agglutinogen A and B) in erythrocytes, and agglutinins (agglutinin a and b) in plasma: Oab (I); Ab (II); Ba (III); They are divided into AB (IV) blood groups. This is very important for clinical hematology and forensic medicine.

Recently, due to the discovery of new agglutinogens, the doctrine of blood groups has become more complicated. For example, group A consists of several subgroups ( $A_1$ ,  $A_2$ ,  $A_3$ ,  $A_4$  etc.).

Determination of blood group is of great practical importance to determine the possibility of blood transfusion. For this purpose, it is important to determine that the donor's erythrocytes do not agglutinate, because the transfused blood plasma does not agglutinate the recipient's erythrocytes due to dilution in the recipient's blood. Only group I blood can be transfused to people of group I. Group I blood can be given to people of all groups. Therefore, people in group I *universal donors* is considered All four groups of blood can be transfused to people of IV group. Group IV blood can be transfused only to people of group IV. People of II and III groups can be transfused blood of the same group, as well as blood of people of I group. Blood of people of group II or III can be transfused to people of the corresponding group and to people of group IV.

When checking the blood groups of the population in different countries, the following average information was obtained about which group people belong to: I

group-40%, II group-39%, III group-15%, IV-6%.

The erythrocytes of most people (85%) contain another factor, which was first discovered by Landsteiner and Wiener in 1940 from the blood of a macaque (*macacus rhesus*) monkey, and therefore it was called the rhesus factor (abbreviated Rh factor). When the blood of a person with this factor (Rhesus positive blood) is transfused into a person who is not characterized by this factor (Rhesus negative), specific agglutinins and hemolysins are formed in the blood of a Rh negative person. Agglutination and severe consequences (hemotransfusion shock) may occur if Rh-positive blood is retransfused to such a person. The development of a Rh-positive fetus in a Rh-negative mother is of particular importance. In this case, the rhesus factor of the fetus passes through the placenta to the mother's blood, as a result, specific anti-rhesus substances are formed in the mother's blood, these substances pass through the placenta to the blood of the fetus by diffusion, agglutinating and hemolyzing its erythrocytes, causing its consequences. Sometimes the stillbirth of the fetus is explained by this.

***Leukocyte*** - is a white blood cell, which is important in the body's protection and recovery processes. Their main functions: phagocytosis; work of antibodies; protein is to break down and remove toxins. In the blood, leukocytes are 600-800 times less than erythrocytes. 1 mm<sup>3</sup> of a healthy person there are 4000 to 9000 leukocytes in his blood. Their increase is called leukocytosis, and their decrease is called leukopenia. Leukocytosis is characteristic of a number of pathological processes (inflammation), but it is also observed in healthy people (during food digestion, physical work, pain, strong emotions, etc.). For example, during difficult exams, it was noted that leukocytes in students reached 11,000.

Leukocytes are divided into two large groups: granular leukocytes (granulocytes) and nongranular leukocytes (agranulocytes). These groups have different origins and functions. Granulocytes (eosinophils, basophils, neutrophils) develop from myeloblasts in the bone marrow. Eosinophils (1-4% of all leukocytes) are stained with acidic dyes (eosin, etc.). They are important in breaking down and detoxifying protein toxins and foreign proteins.

Two immune systems integrally protect the human body: a) cellular immunity in the blood (leukocytes with a segmented nucleus, monocytes, lymphocytes, etc.); b) Antibodies, flocculins, precipitins, etc., which are passed on from generation to generation and increased during life, of the humoral system immunity in the blood.

Cellular immunity in the blood is related to the activity of mature, that is, neutrophils and eosinophilic leukocytes with a segmented nucleus, monocytes in the blood and marrow, and macrophages. The nucleus is a segmented neutrophil leukocyte-round cell with a diameter of 15 microns, when it is stained with panoptic dyes (Pappenheim, Gimza-Romanovsky methods) from pale pink to dark pink, its cytoplasm is dark red-violet. , has a core consisting of three to five, some segments. Some segments of the nucleus are connected to each other by chromatin threads. The cytoplasm of this leukocyte contains about 300 small, powdery cytoplasmic black-gray granules. These particles act as lysosomes in neutrophil leukocytes, as peroxidase, cytochromoxidase and other active substances necessary for cell activity can be seen in them, determined by some specific cytochemical staining methods.

The main function of a neutrophil leukocyte with a segmented nucleus in the human body is phagocytosis (phagos - swallowing in Greek, cytosis - Latin cell), which consists in swallowing disease-causing microorganisms. The immunity of each person is closely related to the activity of segmented neutrophil leukocytes or microphages. Agranulocytosis (final reduction of granulocytes in the blood) caused by external influences or improper use of various drugs leads to an immune crisis, which means illness. Immune crisis causes diseases of internal organs - acute leukemia, hypoplastic anemia, leukopenia, severe infectious diseases with neutropenia, rheumatoid arthritis, etc. It can also cause repeated attacks of some chronic diseases (obstructive bronchitis, pneumonia, cirrhosis of the liver, kidney diseases).

Detection of certain substances in leukocytes helps to diagnose many diseases and predict their development in clinical practice. For example, it is

possible to know the differential difference between safe and dangerous cells in the human body, where DNA (deoxyribonucleic acid) is determined by microspectrophotometry methods; depending on the activity of cytochromoxidase enzyme, it is possible to evaluate the hormonal activity of the thyroid gland; depending on the activity of the peroxidase enzyme, cells belonging to the first granulopoiesis can be identified in the process of blood production in the marrow.

Leukocytes of a healthy person have 23 pairs of chromosomes, of which 22 pairs are called autosomes, and 2 pairs are called sex X and Y chromosomes. Since men have 44 autosomes and two sex chromosomes, X and Y, their karyotype formula is  $44A + XY$ ; karyotype formula of women is  $44A + XX$ . A sex difference was found in neutrophil segmented nuclei of women and men. Sex chromatin ("drumsticks") is found in the nuclei of neutrophil leukocytes with segmented nuclei in women. It is a ball of chromatin with a diameter of 1 micron, which is similar to thread-like chromatin. appears attached to one of the segments. A healthy person's y.s.n. It is 55-67%.

In the blood of a healthy person, In addition to leukocytes, 1-3% of neutrophils with rod-shaped nuclei (so-called leukocytes) are found. This cell with its cytomorphological features is the same as It is a progenitor of a leukocyte, and differs from it in that it has a nucleus that is not divided into segments. T. Ya. a neutrophil leukocyte is a young leukocyte and does not participate in phagocytosis. A segmented eosinophilic leukocyte is a round cell with a diameter of 15 microns. Its pink cytoplasm has a light purple, usually two-segmented nucleus. The segments of the nucleus are connected to each other by chromatin thread. The eosinophil's cytoplasm contains large, round orange-red granules. Eosinophils mainly phagocytose certain cocci and antigen-antibody complexes. In recent years, it has been discovered that eosinophil leukocytes transport components of fibrinolysis. In addition, they are carriers of plasminogen synthesized in the bone marrow. The number of eosinophilic leukocytes in the blood of a healthy person is 0-3%.

A basophilic leukocyte with a segmented nucleus is a round cell with a

diameter of 15 microns. It has a leaf-shaped cell with a pinkish cytoplasm, a nucleus divided into 3-4 segments, and round, large, blue-black grains like shingles. Ya.s. basophilic leukocytes have a unique feature and participate in keeping the blood in a liquid state. In the blood of a healthy person basophilic leukocytes are up to 0.1%.

**Platelet** - the most important, smallest, round, oval, polygonal cell in the blood. Its diameter is 3-6 microns. Platelet consists of two parts, the first part - hyalomer, that is, its small cytoplasm; the second part is granulomer, i.e. grains in the nucleus. Donne (1844) called platelets "globulins", i.e. spheres, while Bizzazero (1882) called them blood "plates", i.e. independent cells. Thrombocyte is the most important cell floating in the bloodstream, because it can be called the "conductor" that controls the blood clotting process. After all, platelets contain more than 60 active substances, 13 of which are directly involved in blood clotting. Thrombocytes are round in a calm state, their cytoplasm is pink, and their granules are dark red. Due to certain effects, for example, under the influence of aspirin, a "release reaction" occurs in them, "antennae" appear at the edges of their cytoplasm.

There are 3 types of grains in granulomas of platelets. Polysaccharides, heparin and other active substances were found in their cytoplasm. The number of platelets in the blood of a healthy person is 180,000 to 320,000.

**Limfotsit** - a round or oval-shaped cell with a diameter of 9-12 microns, dark red-purple color, with a narrow pale or bluish cytoplasm around an eccentrically located nucleus. It was found to contain a small amount of aerophilic granules. It is known that there is a perigranular space around each of these granules. Depending on this feature, lymphocytes can be distinguished from monocytes, which are similar to lymphocytes. Ehrlich gave the name "lymphocyte" to these cells.

Currently, lymphocytes are divided into two types depending on their functions: T and B lymphocytes. Although these lymphocytes do not differ from each other in their cytomorphological characteristics, their functions in the body are different. Lymphocytes make up 25-30% of cells in the blood of a healthy

person, or rather in the formula of leukocytes. Lymphocytes actively participate in the secondary immune response of the human body.

**Monotsit** - a polygonal (polygonal) cell with a diameter of 15-25 microns. In its air-colored or blue cytoplasm, a bean-shaped, dark red-brown nucleus is located in an eccentric position. There are small, red-azurophilic granules in the cytoplasm of the cell. Monocyte participates in the process of phagocytosis in the body. This cell was named "macrophage" by I.I. Mechnikov. Macrophage engulfs foreign cells and their fragments. The activity of these cells is called the primary defense reaction in the body.

In general, monocytes - macrophages perform four different tasks: protecting the body from foreign macro- and microorganisms; interacting with lymphocytes and other antigens in immune reactions; eating weakened, diseased and dead cells of the body; strengthening the regeneration process.

**Plasma cell** - has an oval, oblong, round and polygonal shape. The diameter of this cell is 15-18 microns, and when viewed under a microscope (in smears of blood and marrow stained by the Papengheim or Giemza-Romanovsky method), its bright, dark blue cytoplasm attracts attention. Upon close examination, the cytoplasm is foamy and consists of thousands of tiny secretory lysosomes. At the edge of this cytoplasm, a round, pale purple colored nucleus is visible, and from one end of the nucleus, the cytoplasm is clearly visible in an oval color. Electromicroscopic examination of this area reveals the presence of many mitochondria, which indicates that the plasma cell is an extremely energetic cell. In fact, all immunoglobulins (IgA, IgD, IgE, IgG, IgM) that protect the body are produced in plasma cells and participate in the activity of the humoral immune system as antibodies. They form an antigen-antibody complex by binding foreign, disease-causing antigens, which are engulfed by macrophages and the disease-causing agent is destroyed. The name "plasma cell" was given by W. Waldeyer (1875). 0-2% plasma cells are found in the blood of a healthy person.

**Hemostasis.** Blood provides hemostasis. Blood in a living organism is always in a liquid state. This property of blood provides the opportunity to move

throughout the body and supply it with vital elements. Hemostasis, i.e. the blood clotting system, ensures that blood does not flow out of the body. It is a complex system of enzymes, which includes the blood coagulation system, the anticoagulant system, and the anticoagulant inhibitor system. The process of blood coagulation takes place due to the interaction of active substances in blood serum and platelets.

These substances are the main factor in maintaining hemostasis in blood serum: prothrombin, fibrinogen, blood thromboplastin, calcium ions, proaccelerin, accelerin, proconvertin, antihemophilic globulin, Rosenthal factor, Hageman factor, fibrin strengthening factor.

It is known that according to certain laws, each blood cell has its place in the bloodstream. Erythrocytes flow in the middle of the blood stream, thrombocytes on the edge, and leukocytes in the middle. Platelets produce protocylin (antiaggregant) to avoid sticking to endothelial cells. Nevertheless, the original platelet sticks to the damaged vessel wall. This process is called aggregation. Other platelets stick to it. This process is called agglomeration. Leukocytes in the bloodstream stick to them and form a white thrombi, and erythrocytes stick to them and form a combined thrombi. It activates hemostasis factors and leads to loss of wound healing. As a result, the bleeding stops. Endothelial cells begin to regenerate. When the wall of the blood vessel becomes normal, the unnecessary thrombus is dissolved.

***Color indicator*** – refers to the amount of hemoglobin in each individual erythrocyte and provides information about the hemoglobin inside each individual erythrocyte. This indicator has important diagnostic and prognostic value in medicine. A healthy person's erythrocyte contains 33 µg of hemoglobin. This amount of hemoglobin is one unit (unit) of color indicator. Normally it is equal to 0.9-1.1. Hemoglobin (Greek heima - blood, Latin globuli - ball) is a respiratory pigment with iron in the blood, and in 1864 it was named hemoglobin. Each erythrocyte has about 400 million hemoglobin molecules.

Oxygen entering the body from the external environment, through the

respiratory tract, enters the blood due to the diffusion process through the membranes of the alveoli of the lungs and combines with hemoglobin to form oxyhemoglobin. In turn, it flows with the blood flow to all organs, more precisely, to their cells. Then, due to the low partial pressure of oxygen in the tissues, it separates from oxyhemoglobin and manages to enter the cells due to diffusion. NAD, flavinic acid, cytochromes A, B, C ensure internal respiration of cells. Hemoglobin carries oxygen from the lungs to the tissues, and CO from the tissues<sub>2</sub> delivery to the alveoli depends on its molecular structure.

The hemoglobin molecule consists of two parts, 4% of which is heme and 96% is globin. Gem ( $C_{34}N_{32}THE_4N_4$ ) is a protoporphyrin doped with iron, which consists of protoporphyrin IX and divalent iron. In a healthy body, heme serves as part of the hemoglobin molecule. Globin is a protein part of hemoglobin and consists of four polypeptide chains, two of which are alpha-polypeptide and the other two are beta-polypeptide. Globin consists of 547 amino acids, of which it consists of alpha-polypeptide chains of 141 amino acids and beta-polypeptide chains of 146 amino acids. The last amino acids of the alpha-polypeptide chain consist of valine-leucine, and the last amino acids of the beta polypeptide chain consist of valine-histidine. Therefore, the modern formula of hemoglobin of healthy men and women is NBA ( $\alpha_2\beta_2$ ) is expressed as

In the embryonic period, there are different types of hemoglobin in the human blood, which differ from each other in the property of binding oxygen and some other chemical properties. To identify and separate different types of hemoglobin, the method of measuring the optical density of hemoglobin solutions before and after its denaturation with alkali is used. Different types of hemoglobin are conventionally indicated by the letters NbA, NbG', NbR. NbR hemoglobin is found only in the first 7-12 weeks after the fetus begins to develop in the mother's womb. At 9 weeks, hemoglobin NbG' and adult hemoglobin NbA appear in fetal blood. It is important to note that the hemoglobin NbG' in the embryo can bind oxygen very well and can saturate 60% of the oxygen tension when maternal hemoglobin can saturate only 30%. The structure of hemoglobin is different in

different species of vertebrates. At the same time, heme in different types of globin is the same, and globins differ in amino acid composition.

Three types of hemoglobin are found in the blood of a healthy, adult person: HbA makes up 95-97% of the hemoglobin in the body; HbF makes up 1-1.5% of hemoglobin in the human body; HbA<sub>2</sub> it makes up 2 - 2.5% of hemoglobin in the human body.

According to Max Perutz, who received the Nobel Prize for his services related to the study of the hemoglobin molecule, "hemoglobin molecule can be called the molecular lung of a person" because it supplies the body with oxygen. The main part of hemoglobin is stored inside erythrocytes. A smaller part is in the blood serum. Free Hb in it is normally 0.02-2.5 mg%.

In order to prevent hemoglobin from leaving the blood of a healthy person, it forms a hemoglobin-haptoglobin complex (Hb-Nr) of large dispersion with haptoglobin in the blood serum. Haptoglobin comes from the Greek words "enriching", "retaining", and "globus-ball" in Latin. Hb-Nr is not filtered by the kidneys, so hemoglobin does not leave the body with urine in a healthy person. The amount of haptoglobin in normal blood serum is equal to 50-90 mg%. Haptoglobin, like hemoglobin, is passed from generation to generation. The constant amount of hemoglobin in the blood of a healthy person is kept at 120-150 g/l in women and 130-160 g/l in men.

*Blood plasma composition.* Blood plasma consists of 90-92% water and mainly proteins and salts, 8-10% dry matter. There are several different proteins in plasma that differ in their properties and functional importance: albumins (about 4.5%), globulins (1.7-3.5%) and fibrinogen (about 0.4%). The total amount of proteins in human plasma is on average 7-8%; the rest of the dry matter in the plasma corresponds to other organic compounds and mineral salts.

Nitrogenous compounds other than protein in blood plasma: substances (amino acids, polypeptides) formed as a result of protein hydrolysis and absorbed from the digestive tract and used by cells for the synthesis of protoplasmic proteins, and substances formed as a result of protein breakdown and excreted

from the body (urea, uric acid, creatine, creatinine, ammonia). The total amount of non-protein nitrogen, called residual nitrogen, in plasma is 30-40 mg%. Half of it corresponds to urea. When the kidneys do not work enough, residual nitrogen in the blood plasma increases too much. Nitrogen-free organic matter in plasma: the main source of energy for body cells is glucose (85-110 mg%) and various organic acids, for example, lactic acid, formed as a result of the activity of body cells. Mineral substances in blood plasma make up about 0.9%. They mainly contain K, Na, Mg,  $\text{NRO}_4$ ,  $\text{HSO}_3$  ions are included.

The importance of different blood plasma proteins is different. 1. Proteins create oncotic pressure, which is important for regulating water exchange between tissues and blood. 2. Since proteins have buffer properties, they maintain the acid-alkaline balance of the blood. 3. Proteins ensure a certain level of viscosity of blood plasma, which is important for maintaining arterial pressure at a certain level. 4. Plasma proteins stabilize the blood and create conditions that prevent the sedimentation of erythrocytes. 5. Plasma proteins are important in blood clotting. 6. Proteins of blood plasma are important factors of not suffering from infectious diseases, i.e. immunity.

There are several dozen different proteins in the blood plasma, which form three main groups: albumins, globulins and fibrinogen. Since 1937, the electrophoresis method has been used to separate plasma proteins, which is based on the fact that different proteins have different mobility in an electric field. Blood plasma proteins are mainly formed in the liver. Albumins and fibrinogen are synthesized in the liver. Globulins are synthesized not only in the liver, but also in the spleen, spleen, and lymph nodes, that is, in the organs belonging to the reticuloendothelial system of the body. There are about 200-300 g of protein in the whole blood plasma. Proteins are constantly being synthesized and broken down, so they are rapidly replaced.

**Blood clotting.** Blood coagulation is an important biological defense reaction of the body that prevents blood loss. A blood clot - a thrombus - is formed in the injured place of a small blood vessel, which closes the vessel like a plug and stops

bleeding. When the clotting properties of the blood are reduced, even a small wound can bleed profusely and pose a risk of death. Human blood begins to clot after 3-4 minutes after leaving the vein, and after 5-6 minutes it turns into a completely viable clot. When the inner layer (intima) of the blood vessels is damaged and the clotting properties of the blood increase, blood clots can occur in the veins of the whole body. In this case, a thrombus is formed inside a blood vessel. Blood coagulation is based on a change in the physico-chemical state of plasma protein - fibrinogen. Fibrinogen changes from a soluble form to an insoluble form, turns into fibrin and forms a clot. Fibrin is deposited in the form of long thin threads, forming nets, and shaped elements remain in the loops of the net. When the blood removed from the vein is mixed in the chelchop, most of the formed fibrin remains in the chelchop. Fibrin thoroughly washed from erythrocytes is white and fibrous. Blood separated from fibrin in this way is called defibrinated (defibrinated) blood. It consists of shaped elements and blood serum. Therefore, blood serum differs from plasma in that it does not contain fibrinogen.

## **2.2. Study of the status of detection of agglutinins and agglutinogens in blood stains based on forensic examination materials**

Determining the blood group on new blood stains is not difficult. However, it will not be possible to determine the blood group during the examination of blood stains that have been affected by various external factors and have been formed for a long time. This is primarily due to poor retention of agglutinins in blood stains.

In order to check the ratio of negative results in the detection of agglutinins and agglutinogens in blood stains located on various objects, archival materials of examinations conducted in the years 1992-1996 in the forensic biology department of the Tashkent City Forensic Medical Examination Bureau under the General Bureau of Forensic Medical Examination of the Republic and 2016-2018 at the Forensic Biology Department of the Tashkent Regional Branch of the Republican Scientific and Practical Center of Forensic Expertise archival materials of examinations conducted in the years were analyzed.

Analyzing the archival materials of examinations carried out in 1992-1996 in the Forensic Biology Department of the Tashkent City Forensic Medical Examination Bureau under the General Bureau of Forensic Medical Examination of the Republic, the following was found: after determining the type) 1714 examinations were conducted to determine group membership. The number of checked objects was 4128, and a total of 50873 objects were found in them. When the blood group of all objects with human blood was detected on the stain, 33,357 of them were determined to belong to the group according to the ABO system, which made up 65.6% of the total examined objects. In 16,084 cases (31.6%), separate components of the blood group according to the ABO system were determined. In the remaining 1432 (2.8%) cases, group affiliation was not determined at all, that is, agglutinins and agglutinogens were not found in the blood spots at all. In cases where traces of blood were found on metal objects and in the soil, the blood group was not identified in most cases.

It should be noted that out of 16,084 cases where separate components of the blood group according to the ABO system were determined, A agglutinin was found in 7,709 cases (47.9%), but correspondingly, beta (b) agglutinin was not detected. In 7147 cases (44.4%) B agglutinin was found, but alpha (a) agglutinin was not detected. In 1193 cases (7.4%) agglutinin H was found, but alpha and beta agglutinins were not detected. Among the cases where separate components of the blood group according to the ABO system were detected, the cases where only agglutinins were detected in the blood spots were a deficiency. For example, out of 16,084 cases where individual blood group components were determined, alpha (a) agglutinin was detected in 24 cases (0.15%) and beta (b) agglutinin in 11 cases (0.07%).

When archival materials are studied by seasons, the majority of negative results in determining the group affiliation of blood stains correspond to the first (25.7%) and second (26.5%) quarters of the year, that is, to the winter-spring period. It was found, this figure was 52.2% of all cases. It was found that a small number of negative results (47.8%) corresponds to the third (24.6%) and fourth

(23.2%) quarters, i.e. summer-autumn period.

Analyzing the archival materials of examinations carried out in 2016-2018 in the Forensic Biology Department of the Tashkent Regional Branch of the Scientific and Practical Center of Forensic Medical Expertise of the Republic, the following was found: 526 examinations were conducted to determine group membership. The number of checked objects was 5149, and a total of 8147 objects were found in them. When the blood group of all objects with human blood was detected in the stain, according to the ABO system, group affiliation was determined in 5965 of them, which made up 73.3% of the total examined objects. In 1633 cases (20.0%), separate components of the blood group according to the ABO system were determined. In the remaining 549 (6.7%) cases, group affiliation was not determined at all, that is, agglutinins and agglutinogens were not found in the blood spots at all. In the cases where traces of blood were found on metal objects and in the soil, the blood group was not identified in most cases.

It should be noted that out of 1,633 cases where separate components of the blood group according to the ABO system were determined, agglutinin A was found in 849 cases (51.9%), but correspondingly, beta (b) agglutinin was not detected. In 611 cases (37.4%) B agglutinin was found, but alpha (a) agglutinin was not detected. In 149 cases (9.2%) H agglutinin was found, but alpha and beta agglutinins were not detected.

Among the cases where separate components of the blood group according to the ABO system were detected, the cases where only agglutinins were detected in the blood spots were a deficiency. For example, out of 1633 cases where individual blood group components were determined, alpha (a) agglutinin was detected in 18 cases (1.1%) and beta (b) agglutinin in 6 cases (0.4%).

When archival materials are studied by seasons, the majority of negative results in determining the group affiliation of blood stains correspond to the first (28.6%) and second (27.8%) quarters of the year, that is, the winter-spring period. ri was found, this figure was 56.4% of all cases. It was found that a small number of negative results (43.6%) corresponds to the third (21.2%) and fourth (22.4%)

quarters, that is, the summer-autumn period.

Analytical study over the years showed that group membership was not determined in 31.6% of the examinations conducted in 1992-1996 and in 20.0% of the examinations conducted in 2016-2018. showed that agglutinins were not found in objects. The non-identification of group membership in blood stains due to the absence of agglutinogens in objects was 0.22% in the examinations conducted in 1992-1996 and 1.5% in the examinations conducted in 2016-2018. This indicates that agglutinogens are resistant to external factors.

A large number of negative results in determining blood group affiliation due to the non-detection of agglutinins calls the attention of experts to search for the causes of this condition. For this reason, finding ways to eliminate these shortcomings is an important problem and urgent topic in the forensic examination of physical evidence.

### **2.3. Forensic examination of blood and bloodstain**

When serious crimes are committed, such as murder, defamation, injury, traffic accidents, traces of blood are found on physical evidence, and it is determined to which type and group it belongs. It is very important in solving the crime.

Blood tests are important in the examination of civil cases, for example, in determining paternity and motherhood, in cases of child replacement. In disputed cases, such as establishing paternity and motherhood, when children are exchanged, when comparing the results of physical evidence of the blood of the victim or the accused, liquid blood is tested as a sample for comparison.

In the forensic biology laboratory, blood is examined in the form of liquid and stains (marks).

Individualization of blood is determined by tests on erythrocytes, leukocytes, serum, enzyme systems.

*Erythrocyte system* blood groups are defined in ABO, MNSS, P, Rhesus (Rh), Le (Lewis), Lu (Lassern), K (Kell), Kr, Fu (Duffy), Di (Diego), Hu, I group systems.

**Leukocyte system** more than 10 leukocyte groups (HL-A) are distinguished. The blood test is based on the identification of specific characteristics of antigens present in leukocytes, which give their symptoms from generation to generation.

**Serum system** it is determined by checking the proteins in blood serum (plasma) that give their symptoms from generation to generation. Haptoglobin (Nr), gamma-globulins (Gm), Inv, group-specific component (Gc), lipoprotein (Ag) systems are used in the practice of forensic medicine on the serum system.

**Enzyme system** contains many enzymes stored in erythrocytes and serum of human blood. Isoenzymes are mainly used in forensic medicine. Isoenzymes are catalysts for exactly the same reactions and have the property of transferring their characteristics from generation to generation. Among them, erythrocyte ore phosphatase, cholinesterase, phosphate dehydrogenase enzymes are widely used in the examination of blood stains.

From the above, the ABO system (classical group) is widely used in the practice of forensic medicine due to the fact that it differs from other groups by the presence of not only agglutinogens, but also agglutinins, as well as the use of examination methods. The ABO system was originally founded in 1901 by K. Landsteiner, an assistant at the Vienna Institute of Anatomy, and has not lost its reliability until this time. In the ABO system, the group affiliation of liquid blood and blood spots is checked using several methods for the simultaneous detection of agglutinogens and agglutinins. Simultaneous detection of agglutinogens and agglutinins in blood spots was proposed by N.V. Popov in 1930 and is widely used in practice.

The principles of liquid blood group determination in the forensic biology laboratory are no different from the principles of blood group determination in other medical institutions (surgery, traumatology, blood transfusion stations, gynecology and obstetrics, etc.). These testing methods are based on the determination of the presence of agglutinin and agglutinogens in the blood, and differ only in their technical performance.

If agglutinogens and agglutinins are detected in clinical laboratories using flat

objects (plates, porcelain dishes, plates), blood group is determined by test tubes in a forensic biology laboratory.

***Determining the group of liquid blood by the Schiff crossing binary method.***

Liquid blood to be tested is poured from a syringe (vial) into a chemical test tube. The serum is centrifuged until it is separated from the mass of erythrocytes. Using a pipette, the serum is carefully transferred to a clean test tube. A mixture of 1% erythrocytes in physiological solution is prepared from the mass of erythrocytes. 2 drops of tested blood serum are added to a test tube with 4 drops of 1% Ab(II) group standard test-erythrocyte mixture marked with "A". 2 drops of tested blood serum are added to a test tube containing 4 drops of 1% Ba(III) group standard test erythrocyte mixture. 2 drops of tested blood serum are added to a tube with 4 drops of 1% Oab(I) group standard test erythrocyte mixture. 4 drops of 1% erythrocyte mixture of tested blood are added to a test tube with 2 drops of  $\alpha$ -B isohemagglutinating serum marked with "b". 4 drops of 1% erythrocyte mixture of tested blood are added to a test tube with 2 drops of  $\alpha$ -A isohemagglutinating serum marked with "a". 4 drops of a 1% erythrocyte mixture of the test blood are added to a test tube with 2 drops of  $\alpha$ -(A+B) isohemagglutinating serum marked with "ab". The tubes are centrifuged for 4 minutes. 1 drop from each test tube is dripped into the object glass and covered with a cover glass. It can be seen under a microscope. As a result of the examination, based on the agglutination reaction, the presence of one or another agglutinogen (antigen) in the erythrocytes is determined. If erythrocytes form agglutination due to the effect of anti-B (beta) serum, then it is determined the presence of groups belonging to the A antigen (agglutinogen). Agglutinins (antibodies) are detected in the examined blood serum. If the serum forms agglutination with A erythrocytes, it is determined that the serum contains alpha agglutinin, and if it forms agglutination with B erythrocytes, beta agglutinin is present. The ABO system is divided into four groups. The first group is the presence of alpha, beta agglutinins and antigen O(H) (Oab), the second group is antigen A and beta agglutinin (Ab), the third group is antigen B and alpha (Ba) agglutinin, and the fourth group is only AB antigens (AB). is distinguished by

its existence.

In addition to the classic ABO system, other erythrocyte MN, P, Rh systems are tested using isozyme systems (cholinesterase, or phosphatase, etc.) to carry out disputed paternity and maternity examination.

In the forensic biology laboratory, blood type testing in liquid form is carried out by determining groups of the MN system. Groups of this system include more than ten species. First of all, M and N agglutinogens were discovered in 1927. Then, one after another, other species began to be known. These agglutinogens are present in erythrocytes. This group is called MN system. M and N agglutinogens can be found separately or together in the blood of people. That is why M, N and MN blood types (types) distinguish people from each other. These agglutinogens, unlike the ABO system groups, do not have corresponding agglutinins in the blood. Anti-M and anti-N immunological sera, that is, immune sera obtained by immunization of animals (sheep, rabbit, etc.) are used to determine blood types. The experiment is carried out in flat porcelain plates.

Liquid blood is tested using isoserological system groups (ABO, MNSS, P, rhesus, etc.) in disputed paternity and maternity tests, when a child is changed in a hospital, when a child is stolen, and when a patient needs a blood transfusion. Paternity testing is based on determining the blood groups of the mother, the child and the presumed father. When a child is exchanged or stolen, the blood groups of all family members (father, mother, child) are checked and determined, which depends on the genetic factors of the blood group and deoxyribonucleic acid (DNA). DNA is the genetic code.

The following options are often found in the examination of disputed paternity and motherhood.

Regarding paternity determination (maternity determination) disputed cases:

- this man cannot be the father of the examined child or this woman cannot be the mother of the examined child;

- does not rule out that he may be the father (mother), because the forensic examination of blood cannot resolve disputed paternity (motherhood).

In cases of child replacement or abduction:

- the child cannot be born in the family of P.Abdullaev, but can come from the family of B.Rahimov;

- the child cannot be born in the family of B.Rahimov, but can be born in the family of P.Abdullaev.

Since both children may come from one or the other family, they cannot answer the questions posed by the forensic examination of the blood. Forensic examination determines paternity, motherhood, and origin of a child from a particular family, but does not definitively determine whether it belongs to that family.

The causes of errors in cases of wrong blood transfusion committed during blood transfusion are proved by re-examination of blood groups of the donor or recipient. In conclusion, donor's agglutinogens and recipient's agglutinins are important in blood transfusion. When the blood is incompatible, all red blood cells in the recipient's blood vessels agglutinate and cause death.

**Checking blood traces (stains).** The study of traces of blood is important for the investigation. Depending on their location, number, shape, color, wetness or dryness of the traces, and the level of color saturation, many questions put before the forensic experts are solved. For example, if there is a ring of blood around the corpse, it can be considered as external bleeding. If blood splattered on the walls of the place where the corpse was found, then the blood stain indicates not only the injury in the person's life, but also the bleeding from the artery. The location and shape of the blood traces can also show the position of the corpse at the time of death and the angle of the drop of blood.

Depending on the level of moisture on the surface of the blood traces, the color of the blood stain, it is possible to determine when it appeared, and therefore, on the basis of this, it is possible to say approximately the time of death. From the moment it falls on the product to the moment of inspection, the traces are wet and light-red in color. In this case, external environmental conditions (temperature, season, etc.) are taken into account.

Examining the characteristics of blood traces is not only important in determining cases of death by force, but also in cases of forced sex. For example, depending on the location of the blood stains on the underwear of the accused (suspect), it can be said that they were caused by the victim's dishonor due to forced sex. And finally, studying the external features of blood traces on the clothes and body of the victim, on the clothes and body of the accused and others, helps to determine the ways and causes of their formation.

Thus, studying the characteristics of blood traces can be of great help in solving crimes. It is of great importance to examine the weapons that caused the injury, the places where the accident took place, the examination of the traces of blood in the case of murder, rape, various automobiles and other incidents. Investigating them can play a crucial role in uncovering the truth.

During the examination of blood traces, the following questions are put to the forensic expert:

- is there blood in the stain on the physical evidence?
- who does the found blood belong to: a person or an animal?
- to which group does the blood in physical evidence belong?
- does the blood in the physical evidence belong to a certain person?
- does the blood in the physical evidence belong to a man or a woman?
- does the blood found belong to a child or an adult?
- what part of the body is the blood separated from?
- What is the duration of blood stain formation?
- determine the amount of blood shed as a result of the injury.

The first three of the above-mentioned questions are among the most frequently asked questions in the examination of physical evidence of a biological nature.

**Determining the presence of blood in the examined object.** Determining the presence of blood in individual cases is one of the most difficult problems. Because a lot of substances (various fruit juices, paints, etc.) can look like blood stains when they fall or spill on the surface of various objects, or vice versa,

sometimes blood stains appear on the surface. It may not look like blood at all.

All experiments focused on the detection of blood in traces *approximate (preliminary) and exact (reliable)* consists of verification methods that can be evidence.

***Approximate inspection methods.*** Stains on physical evidence may not always look like blood. Sometimes, depending on the specific characteristics of the item with a blood stain, especially if the traces are located on dark, dark-colored materials, things, it becomes very difficult to identify the traces that cause suspicion of the presence of blood. .

Therefore, in such cases or not, when examining the scene of the accident, it is difficult to distinguish the object of possible blood from the surrounding objects, especially if it is difficult to detect the presence of traces with the naked eye, or blood stains on physical evidence. If they are deliberately lost (clothes are washed or when various fruit juices, dyes are smeared on the stain, and in other cases) preliminary test experiments should be used. They can be used to inspect the scene. In addition, approximate methods of determining the presence of blood are used at the initial stage of the forensic examination to identify spots with a high probability of blood and to conduct the examination more purposefully. These test methods are preliminary steps, as the results of the putative tests cannot clearly indicate whether the stain contains blood or not, and whether the results are positive or negative. At the same time, these tests can help in the approximate identification of physical evidence.

When blood stains are suspected, several preliminary tests are used to detect blood, iron, and blood enzymes. These include hydrogen peroxide, benzidine, luminol, phenolphthalein tests, and ultraviolet light testing.

The initial chemical test experiments developed on the basis of treatment with hydrogen peroxide, benzidine, phenolphthalein solutions were based on determining the presence of catalase and peroxidase, which are blood enzymes.

***Hydrogen peroxide test.*** For this purpose, 2-3 drops of 3% hydrogen peroxide solution are dripped on the suspected blood spot. If there is blood on the spot, a

specific "boiling" of the solution dropped on the spot is observed. Because under the action of catalase and peroxidase enzymes in the blood, hydrogen peroxide breaks down and free oxygen is released and bubbles form on the surface of the liquid.

***Benzidine we have curd.*** For this purpose, when a drop of benzidine and hydrogen peroxide solution is dropped on a stain suspected of having blood, benzidine is oxidized due to the oxygen produced by the decomposition of hydrogen peroxide in the presence of blood, and a blue color appears.

***Sinamated phenolphthalein.*** For this, one drop of a specially prepared phenolphthalein and 3% hydrogen peroxide solution is applied to the suspected blood spot. The appearance of a reddish color within 10-15 seconds is considered a positive result. The appearance of the same color after one minute is not taken into account, because in this case oxidation can also occur in air or under the influence of light.

The presence of catalase and peroxidase enzymes in other fluids of the human body prevents these experiments from being included among reliable test methods.

***Luminol test.*** This screen is used for viewing dark, poorly lit areas. When a luminol solution is sprayed on the stain or a few drops are dripped, a bright air-colored radiation occurs in the presence of blood, which lasts up to a minute.

***Examination under ultraviolet light.*** When suspected blood stains are exposed to ultraviolet light, the blood stain does not fluoresce and appears dark brown in velvety appearance. In this case, the areas around the spot give a certain level of fluorescence. Due to the formation of hematoporphyrin in old blood stains, flame-colored fluorescence is observed in the area of the stain. The test with ultraviolet rays is carried out in the dark. However, rust and other materials look very similar to bloodstains when examined under UV light.

All presumptive tests are non-specific and may sometimes give false positive results in smears containing other substances. Therefore, with these methods, it is not possible to definitively determine the presence of blood in the stains, they only give the possibility to suspect the presence of blood in the stain.

**Reliable test methods.** In the practice of forensic medicine, the following reliable methods are used to determine the presence of blood in a stain on physical evidence:

- methods of morphological examination;
- spectral inspection methods;
- reactions of microcrystals;
- chromatographic testing methods.

***Methods of morphological examination*** it is based on the detection of blood form elements, hemoglobin or its derivatives (derivatives) using a microscope, and is considered one of the most reliable methods for determining the presence of blood in a stain. This method gives a positive result only if the blood is liquid or newly formed blood spots. However, the morphological method is not widely used in forensic medicine laboratories, as the shape elements of blood, after falling into the external environment, twist, change their shape and characteristics, and it becomes impossible to identify them.

***Spectral inspection methods*** absorption method of microspectral analysis carried out using a microspectroscope is based on the determination of hemoglobin and its derivatives in a blood spot. For this, a drug (cloth fiber or a small particle of an object containing a stain) is prepared from the examined stain and treated with appropriate solutions. If there is blood in the stain, absorption lines (spectra) are formed based on the ability of hemoglobin derivatives (hemochromogen, hemotoporphyrin) to absorb light. A small amount of object is required to check the presence of hemoglobin and its derivatives in blood spots.

In 1965, the Russian scientist M.A. Vasilev, by examining a number of chemical elements in the blood, put into practice the method of determining the presence of blood in the case of deep decomposition of blood, for example, when textile tissues are burnt to coal, using emission spectral analysis.

***Reactions of microcrystals*** based on finding hemin and hemachromogen crystals in the examined object. The microcrystal method of determining the presence of blood on a stain is observed when the object under investigation is

exposed to solutions of a specific color and shape. For example, sodium chloride and acetic acid are added to a test tube containing fabric fibers or stain particles, and then heated. If there is blood in the stain, crystals of hemin substance are formed. When they are examined under a microscope, they appear in the form of a brown oblique parallelogram. Later, it was found that hemin crystals can be separated from other halides (iodo-hemin, bromhemin, etc.).

Positive results of spectral and microcrystal tests indicate the presence of blood in the stain, but even the best test cannot show its absence. Because many factors of the external environment can have a bad effect on these experiences (reactions). If blood traces are contaminated with paints, cement, soil, lime, oil, fuel oil, gasoline, kerosene, other fuels, soap, rust, gung and other substances; when spots harden; when there are changes caused by decay; when exposed to high temperatures; if it has lost its properties due to sunlight, detergents and several other factors, then microcrystals and spectral reactions will not give good results. Finally, absorption lines (spectra) characteristic of hemoglobin derivatives (hemochromogen) can also be formed when examining traces that do not belong to blood. For example, cytochromes widely distributed in nature can react positively.

***Chromatographic testing methods.*** The method of chromatographic examination was proposed for the first time in 1903 by the Russian scientist M.S. Tsvet. Scientific works of M.S. Tsvet served to develop other types of chromatographic tests.

Many factors of the external environment: when traces of blood are contaminated with oil paints, cement, soil, lime, oil, fuel oil, gasoline, kerosene and other fuels, soap, rust and other substances; when the spots are hardened; when there are changes caused by decay; when exposed to high temperature; under the influence of sunlight; microcrystal and spectral reactions do not give good results when exposed to synthetic detergents.

In the cases mentioned above, the use of chromatographic testing methods gives a positive result. One of the most common forms of chromatography is paper chromatography. The above-mentioned soil mixture, cement, gung, rust, ganch,

lime, sand, chlorophyll, fuels, paints, etc., have their own effect on the chromatograms of blood stains according to the purification and separation properties of chromatography. does not pass the secret. This method is one of the most common methods. According to the execution technique, it is divided into paper or thin-layer chromatography. The first of these is the easiest to do. It is very easy to perform and allows you to get a number of analyses.

***Determination of the presence of blood on a stain by the method of chromatography on paper.*** First, chromatographic paper is prepared for the experiment, 1 cm wide lines are drawn on it with a graphite pencil, and the starting line is marked at a distance of 2 cm from the lower edge. Along both sides of this line, parallel cuts of 0.3 cm are made in each direction, the distance between pairs of cuts should be about 0.4 cm. Fragments (threads) of the stain to be examined, the carrier of the object and the known blood sample are placed on the prepared incisions. Chromatographic paper with a test spot, object carrier and fragments (strings) from a certain blood sample is placed at the bottom of the glass chamber with a solvent. After the solvent reaches the upper edge of the paper, the paper is removed from the glass chamber, the solvent level is marked with a regular (graphite) pencil, and it is dried in a fume cupboard at room temperature. The dried chromatographic paper is first treated with a 0.1% solution of benzidine in chloroform and then developed with a solution of 3% hydrogen peroxide. Chromatographic paper is washed under running water. After exposure of the chromatogram, the blood pigment area is immediately stained blue, and after washing under running water, the blood pigment area becomes a stable reddish-brown color that does not change against the background of the flow of the paper. Such color changes confirm that the stain is blood. If the area of blood pigment in a given blood sample immediately turns blue and after washing under running water becomes a stable reddish-brown color with an  $R_f$  of 0.11 to 0.15, the subject If the carrier sample does not stain, the reaction is considered positive.

***Determination of the presence of blood on a silofol plate by the method of microchromatography.*** To conduct the experiment, first, a cellophane plate is

prepared, with the help of a scalpel or sharp tweezers, 1 cm wide strips are made on the cellophane plate, at the same time, the upper layer of the aluminum plate is cleaned. At a distance of 1.5 cm from the bottom edge, a starting line is marked with a graphite pencil, and along this line, the plate is bent without damaging the outer layer. On the prepared starting line of the plate, the stain of the object to be examined, the carrier of the object and the known blood sample (or the samples are layered with the help of glass capillaries) are fixed in the appropriate way with a pencil. The Silufol plate and the test object installed on it, together with its object carrier and pieces of the known blood sample (or object pulls), are carefully placed at the bottom of the Petri dish with a universal solvent, closed with a lid. When the solvent reaches the top edge of the plate, it is removed, +100 for 15 minutes<sup>0</sup>C is placed on the thermostat. The heated plate is placed alternately in a Petri dish with a developer and then in a Petri dish with 3% hydrogen peroxide. When the chromatogram is displayed, depending on the concentration of the blood in the test object, a blue color is formed from the starting line to the finishing line. If the pigment area in a certain blood sample is intensely blue, the reaction is considered positive.

Affine chromatography is one of several methods of checking by chromatography method. Affinity chromatography test method is designed to concentrate alpha and beta agglutinins in blood diluted in a physiological solution. It is confirmed by the data of scientific literature that the precipitation of serum globulins on the surface of antigen in highly diluted serums is higher than in low-diluted and undiluted serums.

Affinity chromatography method provides detection of blood agglutinins of the ABO system in weakly expressed blood spots contaminated with various substances. This method is simple and easy to implement. Also, the affinity chromatography method is fully suitable for the detection of isoantibodies in blood stains located on various textile fabrics.

**Determining whether the blood on the stain belongs to a person or an animal.** Since ancient times, people have been interested in determining whether

blood belongs to a person or an animal. For this purpose, the size of erythrocytes was measured, the shape of hemoglobin crystals was studied, an attempt was made to use the difference in the degree of alkaline denaturation of blood, etc. However, all these methods are generally unreliable and have not been put into practice. Only in 1899, after the discovery of the specific type of antibody by F.Ya. Chistovich, a clear and relatively uncomplicated method of the relationship of blood to types in forensic medicine *precipitation reaction* appeared. In 1902, the German microbiologist Ulengut found that specific antibodies are related to humans. That is why this reaction is called the Chistovich-Ulengut experiment after the scientists who discovered it.

When blood is found in the stain, it is necessary to decide whether it belongs to a person or to what kind of animal. Such an examination is of great importance in determining whether the blood found in physical evidence belongs to a person or an animal. Because in many cases, the suspect denies that he is involved in the crime and shows that the traces of blood stains belong to the animal and were formed during hunting.

In the experience of forensic science, blood types are determined by an immunological (using sera) method, more precisely, by a precipitation reaction. The precipitation experiment does not detect the blood type (types), but rather the type of protein. Because good results in experiments can be obtained not only with a certain type of blood, but also from the body's sperm, skin, tear fluid, urine, saliva and saliva.

Precipitation serum is obtained by immunization of animals. In most cases, rabbits are immunized. If a rabbit is injected with human blood serum, its body produces antibodies (precipitins) against this protein, and this rabbit serum is called human protein-precipitating serum. In the same way, sera of cattle, dogs, horses, pigs, birds and hawks were obtained by protein precipitation. They should be clear and transparent.

Determination of blood types is performed in two variants - liquid and thick (gel). The first of them is widely used in practice.

***Precipitation reaction in a liquid medium by the Chistovich-Ulengut method.*** In order to determine the type of blood in the traces, a strained liquid is prepared from them with the help of a physiological solution. Then dilute the sample 1:1000 and, if there is a fresh stain (about straw yellow in color) with the protein of the more suspect human or animal species, slowly overlay the serum. in the form of This experiment is carried out in special cone-shaped test tubes with a narrow bottom. They are also called Chistovich-Ulengut test tubes. If a white, round-shaped precipitation line is formed between the serum that precipitates the human protein substance and the liquid obtained from the stain, then the blood stain is considered human.

If it is suspected that the examined objects contain the blood of an animal, for example, a large horned cattle, a dog, a pig, or a hawk, then the precipitation reaction is carried out with the help of sera that precipitates the protein of these animals.

The precipitation experiment is carried out not only in liquid form, but also in an agar layer. Molten agar is poured in a thin layer on the glass slide. After the agar layer hardens, grooves are formed in it. For comparison, the carrier (from the left) and the samples taken from the stains (from the right) are poured.

Three more separate wells are built around the middle wells, and in their wells are sera precipitating human protein (Od) and two different protein precipitating sera, for example, poultry (P) protein, bovine (QM) protein Precipitating specific immune serum is injected. Serum (antibody) and suspension (antigen) fluids are absorbed into the agar layer and meet each other. If the antibody and antigen are homologous (the same), a white precipitate line will form between them. If the antigen (gravity) and antibody (serum) are heterologous (different), then this line will not be formed.

The experiment of precipitation in agar layer can be carried out by another, improved method. A layer of agar is prepared by adding human or other protein precipitating serum to agar liquid. After hardening, round holes are carved into it. Extracts from the blood stain and clean tissue around the blood stain are poured

into these pits. Therefore, if a circle of white precipitation appears around the well in the layer (in the process of diffusion), then this sample indicates the presence of a homologous protein substance in that precipitating serum. For example, if the precipitating circle is prepared from bovine precipitating serum solution, it will indicate the presence of bovine blood in the stain. Therefore, if the precipitation of this circle is formed on an agar layer composed of liquid human serum, it indicates that the stain belongs to human blood. A white precipitation circle (ring) does not appear around the strainer made of a carrier (clean) fabric.

***Determining the type of proteins in fragments of muscle, bone and organs on agar.*** Initially, precipitating sera with a titer of not less than 1:10000 are selected for the experiment. Then the muscles, bones and organs to be reacted are marked and crushed. A groove is made on the agar prepared in a Petri dish with the help of a punching device: the number of peripheral grooves depends on the number of objects, and as a control, the extracted physiological solution and antigen should be added to the reaction. 1-2 drops of precipitating serum are poured into the central well, pieces of crushed muscle, bone or organs are placed into the peripheral wells, and 1-2 drops of physiological solution are dripped over it. The Petri dish is closed and the moist chamber +4-6<sup>0</sup>It is placed at a temperature of C. Monitored periodically for 24-72 hours. If a precipitation line is formed between the test objects and the grooves into which one of the precipitating sera is poured, the species is determined. If a positive result is not obtained with the sera used in the reaction, all precipitating sera available in the laboratory should be added to the reaction. If the precipitation lines are formed between the grooves of the precipitating serum and the corresponding antigens, the reaction is considered to have been carried out properly.

**Determining the group belonging to the blood found in the stain.** If human blood is found on the stain, it is necessary to determine to which person the blood belongs. It is important to determine the group of blood, that is, it is determined whether the blood found on the clothes and body of the suspect belongs to the victim or not. Based on the result of this investigation, it is decided whether the

person is guilty or innocent. In order to solve this question, isoantigens are checked on a stain from human blood. Meanwhile, isoantigens of erythrocytes, serum, leukocytes and enzymes are differentiated. Among them, the first and second series are used more often in the practice of forensic medicine.

Therefore, the difference between the blood of one person and the blood of another person depends on the immunobiological properties of the substances in the blood. Their identification begins with the first examination of the ABO erythrocyte system. The ABO system is divided into IV group. If the blood of the person found in the physical evidence matches the blood of the two suspects tested in the case, that is, they are the same (according to the ABO system), then the blood groups belonging to another system, for example, MN groups, are tested. If it is not differentiated by this system, then the P system or other systems (including the serum system if deemed necessary) are examined.

After testing the blood that was submitted (or taken) for the sample, the expert examines the blood in the physical evidence and compares it with the sample (previously known) blood. If the blood found in the physical evidence does not match the blood of the suspect, the expert can answer that the blood found is not his blood. But in the case of matching with his blood, the blood found in physical evidence cannot be said to be the blood of this person, because the blood of other persons may also belong to this group. Therefore, the expert cannot deny that the blood found in the physical evidence is from the blood of another person. Of course, the more antigens are tested, the more reliable the result.

Detection of antigens belonging to the erythrocyte system in a blood spot consists of several methods, the most commonly used of which are: absorption of agglutinins, absorption-elution and mixed agglutination methods.

Group affiliation of the blood stain *agglutinin absorption method* It is based on the reduction of the titer by separately treating the blood spot with specific titer alpha (anti-A) and beta (anti-B) agglutinins for 18-24 hours, in which it is determined to which group the antigen of the blood belongs. For example, if the initial titer of alpha serum is equal to 1:32 and after absorption corresponds to 1:4,

and the titer of beta serum does not change, then the human blood found in physical evidence is considered to belong to antigen A. The disadvantage of the agglutinin absorption reaction is that it requires the use of a relatively large amount of blood stain.

In later years *absorption-elution reaction* widely spread in practice. This method provides an opportunity to detect the antigen in a blood-soaked thread 4-5 mm long and is based on the reversibility of the antigen-antibody reaction. At low temperature (0°(close to C) absorption of blood spot antigen with standard serum antibody occurs. Then +56°At a temperature of C, the bound antibody is separated using an isotonic solution of hydrochloric acid, where it is determined together with standard erythrocytes. If there is no antigen on the stain, the reaction is relative. Although the rule is simple, this reaction is very difficult to perform and can only be performed by highly qualified experts.

***Determination of the group of the ABO isoserological system in small blood spots by the absorption-elution method with the help of α-H (herbaceous elder) extract.*** To conduct the experiment, separate threads measuring 0.2x0.2 cm or 0.5-0.6 cm long are cut from the blood stain and object carrier to be tested, as well as from a blood sample of a certain group. The slots of the tablet are marked: "K ob. №..., ob. №..., KD, A, B, AB, O". Each object is placed in designated agglutination test tubes and 2 drops of α-H elderberry extract are added. Test tube tripod +4-6°C is placed in the refrigerator for absorption for 20-24 hours. The job log shows the start time of the reaction execution. Objects from serum tubes are removed onto filter paper using a closed-ended pipette. A deep-grooved tablet and a chilled physiological solution are placed in a special container with ice. For each object, depending on the titer of the extract and the effect of the object carrier, 2-3 rows of grooves are filled up to 2/3 with chilled physiological solution. The objects to be examined are taken using thin-tipped tweezers and placed in the first row of grooves under the control of a 2-minute timer. After washing, the objects are transferred to filter paper and dried. Clean test tubes are marked and dried objects are placed in them using tweezers. 2 drops of physiological solution are dripped

into each test tube. Test tube tripod +56<sup>0</sup>C is placed in the thermostat for 30 minutes. Threads (particles) in test tubes are removed on filter paper using a closed-ended pipette. 1 drop of 1% erythrocytes of Oab(I) group is added to test tubes marked with "a-H". Centrifuge at 1500 rpm for 4 minutes. Shaken. 1 drop from each test tube is dripped onto the microscope slide, a coverslip is placed over it and viewed under a microscope. In cases where agglutination is observed in the test tubes of object carriers, it is necessary to carry out measures to eliminate the effect of object carriers. If agglutination is not observed in the test tubes of test objects and their object carrier weights, the reabsorption-elution reaction should be carried out starting from the absorption phase. The absorption phase is carried out several times (sequential) absorption): threads of objects are poured with serums for 2 hours, serums are removed with a pipette, again poured with serums for another 2 hours. After this manipulation is carried out several times, a complete absorption reaction is carried out with the last portion of the serum for 20-24 hours. If agglutination is observed in the series of test tubes marked with "α-H", it indicates that the N antigen has been detected. If no agglutination is observed in the test tubes with the object carrier, and agglutination is observed with the corresponding erythrocytes in the blood samples of a certain group (i.e. between α-H herb elderberry extract and O), the reaction was carried out appropriately and the blood group was determined. is considered

Another similar one in expert practice "*mixed agglutination*" reaction began to be used. The essence of this method is that the antibody of the standard serum binds both to the antigen of the blood spot and to the standard erythrocytes under certain conditions. Therefore, when there is an antigen in the stain, erythrocytes "coral" and agglutinants are observed in the blood stain.

Although the absorption-elution and mixed agglutination methods require a small amount of material, both of these methods are used in practice for the examination of small blood spots due to their complexity and the need for many tubes.

To eliminate the above-mentioned shortcomings, at the same time, to

determine the antigens of the ABO system *affinity chromatography method* recommended. This method is one of the methods of chromatography examination, and due to its ability to clean the contaminated object, sensitivity and simplicity, it is important in forensic medicine. The essence of this method is that the fiber taken from the blood stain is clamped to the starting line of the chromatographic paper, the paper is placed in the chromatographic chamber, and infinitely diluted anti-A and anti-B sera based on the property of capillary absorption over the stain will be held. As a result, the antigen pulls only its homologous antibody from the liquid. If there is a heterologous antibody in the absorbed liquid, then it will pass directly from the blood stain to the embedded fiber without being absorbed by the liquid stream. The advantage of this method is that it is possible to check several objects at the same time. In addition, it is possible to determine antigens belonging to the ABO system even in the objects where the presence of blood was detected by the chromatography method.

Determining the blood group in new blood stains is not difficult. However, it will not be possible to determine the blood group during the examination of blood stains that have been affected by various external factors and have been formed for a long time. This is primarily due to poor retention of agglutinins in blood stains.

As a result of the conducted scientific research, it was possible to detect agglutinins by first treating the blood stains of the hemolyzed and decomposing corpse with ethyl alcohol.

Also, in order to improve the detection index of agglutinins in corpse clothes with blood stains, it was suggested to dry them in high-temperature dry air beforehand.

In blood spots contaminated with various substances (soil, sand, gasoline, fuel oil) or with a low titer of agglutinins, blood spots in blood spots using affinity chromatography (biospecific adsorption chromatography) even in cases where it is not possible to determine group affiliation using existing methods It has been proved by laboratory tests that agglutinins can be determined by concentration.

**Serum systems.** In addition to the erythrocyte system, the serum system is

also checked in the blood spot. Serous systems are hereditary systems. Haptoglobin and gammaglobulin systems are often tested from this system. When studying the protein components in human blood serum, it was observed that there is a difference in the serum protein of different people. It turns out that all people can be divided into groups based on serum protein and erythrocyte antigen. The polymorphism of serum proteins is characterized mainly by different electrophoretic mobilities, different antigenic properties, and sometimes a combination of both. Currently, serum immunoglobulins (Gm, InV or Km), haptoglobin (Nr), group-specific components (Gc), lipoproteins (Ag, Lp, Ld), protease inhibitors (Pi), transferrin, various complement components (S3, S6) and others are well studied.

Examination of the serum system with the erythrocyte isoserological system can be useful in resolving the question of whether or not the blood stain is from a specific individual. The immunoglobulin system associated with Gm and InV antigens is especially important. In recent years, special attention has been paid to the determination of the haptoglobin group from blood stains in expert practice.

Haptoglobin, like hemoglobin, is hereditary. According to the haptoglobin system, all people are divided into 3 groups. No. 1-1, No. 2-1 and No. 2-2. These are determined using starch, agar-starch and polyacrylamide electrophoresis. Polyacrylamide electrophoresis gives very good results. In this case, a good result is observed even if the duration of the blood stain is up to 1 month. Gammaglobulin (Gm) is divided into more than 20 groups. Also, Gc, that is, group-specific components, is determined.

Examination of haptoglobin of living persons and freshly dead corpses does not present any difficulties. However, long after death, the haptoglobin groups become difficult or impossible to detect due to hemolysis, suppuration, thickening, turbidity of the serum or complete separation of the blood, and other reasons. Taking into account the above, it is important to find methods for determining the haptoglobin group in the blood of a hemolytic, festering corpse. To achieve this goal, the presence of serum haptoglobin in precardiac, pleural and abdominal

cavity fluids was checked. As a result of the experiment, it was proved that the group of haptoglobin in the mentioned fluids corresponds to the group of haptoglobin in blood serum. When the fluids of the pleural and abdominal cavities and the fluid of the pericardial sac were examined in parallel, it was found that the speed of haptoglobin fractions in them is different, the speed of the haptoglobin fractions of blood serum and pericardial fluid is the same, but sometimes it is faster in the pericardial fluid than in the blood serum.

Experiments have shown that the haptoglobin fraction in the pleural and peritoneal fluids is much lower, and in turn, the haptoglobin fraction in the peritoneal fluid is much lower than the haptoglobin fraction in the pleural fluid, and in comparison with the pleural and peritoneal fluids, in the pericardial fluid showed that the serum-specific haptoglobin group has a high intensity.

Therefore, in equal circumstances, when determining the haptoglobin group in the blood of a hemolyzed, purulent corpse, it is necessary to check the pericardial fluid, and only in cases of its absence (when the heart, pericardium is injured, etc.), it is necessary to check the pleural cavity or abdominal fluid. Based on the above, haptoglobin phenotypes can be determined by examination of pericardial fluid in hemolyzed, suppurating cadaveric blood.

**MNSs isoserological system.** In 1927, antigens not associated with ABO blood group were found in human erythrocytes. These antigens were called M and N. There are 3 forms of joining them together (M, MN, N), and based on them, the blood of all people is divided. Later, additional S and C antigens were discovered in this system, increasing the number of these groups to 9. It turned out that it is even more difficult to carry out inspections in this system. Difficulties in detecting M and N antigens in a blood spot using a quantitative absorbance reaction or a highly sensitive absorbance-elution reaction are observed, since the M antigen sometimes binds non-specifically to anti-N antibodies. That is why M and MN groups in the stain are difficult to compare. Therefore, this system can be used when M antigen is present in one part of the site and not in another. It is possible to compare people into these 2 groups based on blood stain with sufficient accuracy.

**P isoserological system.** In 1927, antigen P was found in erythrocytes, and all people were divided into 2 groups based on the presence or absence of this antigen. Currently, 5 groups have been identified in this system:  $P_1$ ,  $P_2$ ,  $P_1^x$ ,  $P_2^x$ , P. Only one P at the disposal of the expert<sub>1</sub>- has anti-serum.  $P_1$ -antigen is less resistant and can be found in blood stains that have been formed for a short time. Absorption-elution reaction is usually used for this purpose.

**Rhesus isoserological system.** In 1940, an antigen belonging to the blood of a macacus rhesus monkey was detected in the erythrocytes of many people. That is why it was called the Rhesus factor. Based on the presence or absence of such a factor, all individuals can be divided into 2 groups.  $Rh_{0+}$  (D+) goes  $Rh_{0-}$  (D-) or rh (a). Further examinations of the rhesus factor D, S,  $S^{same}$ , He showed that there are E,  $\Delta$ , C, E and other types. Rh in the blood on the spot<sub>0</sub> (D) to identify the antigen in 1974 Galtseva Ye.Ye. The revised type of absorption-elution reaction proposed by

**Other isoserological systems.** The discovery of the Rhesus factor led to a strong investigation of antibodies that appear during re-transfusion, as well as during pregnancy in women. In this way, Lasereen, Lewis, Kell, Duffy, Kidd, Diego isoserological systems were opened. These systems are named after the person who first discovered the antibody against the antigen. Among these, the most important for forensic medicine is the Lewis system. In this case, antigens are found through quantitative reactions of absorption-elution and absorption, which can be determined in a blood spot.

**Australian antigens (HB antigens).** In 1965, an antigen was found in the blood serum of a person living in Australia, and later it was identified in other people. It is known that it is spread in 1-2% in middle latitudes, and up to 10% in hot countries. Unlike other serum antigens, Australian antigen is not transmitted from generation to generation because it is linked to the hepatitis V virus. Antigen resistance in a blood spot lasts up to 5-7 months. That is why it is used to determine the possibility of the formation of traces of blood from one or another person. In 1981, Kisin M.V. and others have created a method for simultaneously

finding hepatitis V antigen and determining blood groups.

**Blood enzyme systems.** In 1957-1959, many erythrocytes and human serum enzymes were found to have the character of genetic polymorphism. It has been proven that many enzymes are found in the form of isoenzymes in most people. They accelerate the same reaction, but differ in electrophoretic activity in their fractions. Erythrocyte enzyme systems, including acid phosphatase, phosphoglucomutase, adenylate kinase, adenosine deaminase, glutamivatamino-transferase, 6-phosphogluconate dehydrogenase, esterase D and others are important for forensic medicine. All people can be divided into groups according to each isozyme system, like isoserological, serum and leukocyte. In forensic practice, isoenzymes are currently detected only in some laboratories.

**HLA leukocyte antigen system.** It is a genetic system that contains about 90 different antigens. They are found in the membrane of all nuclear-retaining cells of a person and play an important role in organ and tissue transplantation (transplantation) due to the presence of antigens that determine the correspondence of tissues to each other. The discovery of genetic antigen patterns made this system possible to use in the controversial childhood diagnosis.

**Determining the gender of the blood stain.** Determining whether a blood stain belongs to a man or a woman (individualization) is of great importance in the practice of forensic examination. A series of tests will be conducted for it:

*Determination of sex by chromosomes.* Males have only one genetically active X-chromosome. Therefore, theoretically X-chromatin may not be found. In humans, the Barre corpuscle is easily found in the epithelium of the mucous membrane of the oral cavity. In women, the cell with X-chromatin is 20-80 percent, and in men it is 0-4 percent.

*Detection of X-chromatin in somatic cells.* M. Wagg and Y. Bertram (1949) examined the male neuron of a cat for the first time in all mammals, as well as in humans, female-specific chromatin was identified. This chromatin is a particle about 1  $\mu\text{m}$  in size, and it is more intensively stained with the main nuclear dye than other nuclear chromatins. Then to such a derivation *Barre body* is called

Usually, they are located on the inner surface of the nuclear membrane in a triangular, millet-like, trapezoidal shape, sometimes thickened or toothed in the nuclear membrane. The origin of the Barre body has been determined in the present period. In the somatic cells of women, one of the X-chromosomes is in an active state, and the other is genetically passively spiraling into a hyperchromic state and can be found in the form of X-chromatin.

***Determination of X-chromatin in leukocytes.*** When determining sex chromatin in blood, many researchers tried to find Barre bodies in leukocytes. This, in turn, led to the opening of morphological structures in the shape of a characteristic bulge, which has a sexual character, in the segment of the nucleus of neutrophils, eosinophils and basophilic leukocytes (W. Davidson, D. Smith, 1954). The A-type hump (drum stick), which is more typical for the female sex, looks like a hanging drop. The thickened part has a homogeneous structure and is stained more intensively than the core. It can be seen that it is connected with the nuclear segment by a thin leg. The size of such bumps is 1.5-2  $\mu\text{m}$ , and they are 10-12 times smaller than the nucleus. In type A, the buttocks are observed only in women. In males, similar structures are observed, and they are found to be small in size and weakly painted. For women, the specific B-type bump (knot) has the same dimensions and colors as the A-type, but it is much shorter and has a thicker leg. They are less common in men. C-type nodules are of various shapes (small rod, stick, small lump, racket, filamentous growth, tube-like structure, etc.), less than 1  $\mu\text{m}$  in size, and weak. It doesn't look flirtatious or sexual.

In practice, only A and B type bumps are taken into account in segmented leukocytes. They are considered the equivalent of sex X-chromatin in leukocytes. Y-chromatin. T.Caspersson and others (1969) found that after staining the Y-chromosome with derivatives of acridine (acrychin or atebrine, acrichin-musp) in the lower part of the long shoulder of the male chromosome, a bright fluorescence appears.

Later, P. Pearson et al. (1970) found bodies 0.3-0.7  $\mu\text{m}$  in size after staining cells with atebrin in the interphase nucleus during the resting period using

ultraviolet light. This body is Y-chromatin and is observed only in humans and monkeys. Y-chromatin is round or sickle-shaped, clearly visible, located at the base of the nuclear membrane, as well as in the karyoplasm. According to various authors, Y-chromatin is observed in all organs and tissues and leukocytes of men from 20 to 99 percent.

**Determining whether the blood on the stain belongs to a newborn or an adult.** The need to determine whether the blood on the stain belongs to a newborn or an adult arises in the investigation of child murder crimes. Hemoglobin in the blood of newborn babies differs from hemoglobin in the blood of adults in terms of physico-chemical, biochemical, and immunological properties. This was the basis for dividing hemoglobin into two types. 1. HbF - fetal type hemoglobin in the baby's blood. 2. HbA - hemoglobin of an adult. These two types of hemoglobin are analyzed using an electrophoresis reaction. HbF differs from hemoglobin, HbA-hemoglobin by its high resistance to the effects of alkalis and acids, high antigenicity and oxidizing ability. The amount of HbF in the umbilical cord blood of a full-term fetus is equal to 80%. As the child grows, the amount of HbF hemoglobin in the blood decreases. By the time a child reaches 1 year of age, the amount of HbF is around 1-4%. It should be noted that the amount of HbF in the blood of older people can increase during pregnancy and in a number of pathological conditions. With the help of microspectral examination method, it is possible to determine the level of alkali resistance of hemoglobin from human blood. Compared to HbA-hemoglobin, it was found that HbF-hemoglobin in the blood of older people is more resistant to the effects of alkali.

**Determining which part of the human body the blood flows from.** From which area of the human body the blood in the examined object was formed as a result of the flow is determined depending on what additional elements are in the blood stain. The presence of epithelia of the mucous membrane of the respiratory tract in the blood stain indicates that the blood has flowed from the respiratory organs. The presence of stool elements in the blood stain indicates that the blood is rectal hemorrhoidal blood. The presence of endometrial epithelium proves that the

blood is menstrual blood. If there is no additional element in the blood, it is impossible to determine where the blood came from.

Several methods have been proposed to determine whether a blood stain is caused by menstrual blood. These methods are methods for determining the presence of cells of the uterus and vaginal mucosa in the stain and for determining the activity of the diamine oxidase enzyme.

Currently, a method based on the expression of LDG-4 and LDG-5 isoenzymes is used to determine whether the stain is from menstrual blood. The amount of these isoenzymes in menstrual blood is higher than in blood in peripheral blood vessels. Over time after the formation of a blood spot, the amount of these isoenzymes is expressed differently in spots formed from menstrual blood and blood from peripheral blood vessels. If the amount of LDG-4, LDG-5 isoenzymes is low in the blood spot in the peripheral blood vessels after 1-2 days after the formation of the blood spot, after 1 week in the stain they disappear completely. The activity of these isoenzymes in the stain formed from menstrual blood remains up to 1.5 months and allows detection. It can also be determined that the blood stain is caused by menstrual blood by examining the protein (pp-12) in the lining of the uterus. The amount of this protein in menstrual blood is 2000 times higher than in peripheral blood.

**Determining the time of formation of a blood stain.** One of the methods of determining the time of formation of a blood spot is based on checking the activity of cholinesterase, leucine aminopeptidase and oxytocyanase enzymes in the spot. The cholinesterase enzyme in the blood serum can be stored in the blood spot for 3-5 months, the leucine aminopeptidase enzyme for 50-60 days, the oxytocyanase enzyme for 80-100 days, and the oxytocyanase enzymes.

Another method of determining the period of formation of a blood stain, which is widely used in the practice of forensic medicine, is based on the spectral examination of the transition of the hemoglobin substance in the blood to derivatives (hemoglobin derivatives). In this method, hemoglobin, oxyhemoglobin and methemoglobin spectra are checked.

There is also a method for determining the duration of the blood stain based on the distribution of chlorides contained in the blood stain to the stain-preserving material. After the blood falls on an object, the chlorine ions in the blood pass into the surrounding tissue and form a border around the stain. The longer the spot, the wider the border around it. The presence of chlorides in a blood stain is determined using 1% silver-nitrogen acid. The width of the formed chlorine border indicates the time of formation of the stain. Chlorine absorption of a blood stain depends not only on the time of the stain's formation, but also on environmental influences, so this method of examination is of little importance in the practice of forensic medicine.

**Determining the amount of blood shed.** In the course of the investigation, questions may arise as to whether the death occurred in the place where the body was found or whether the body was brought from another place. These questions are solved by determining the amount of blood spilled around the corpse. There are several ways to determine the amount of blood shed, the most important of which is to calculate the amount of blood shed by determining the weight of dried blood. The error of this method is 15-20%.

### **Control questions**

1. What methods are used to determine the presence of blood in a stain on physical evidence?
2. What are the reliable ways to determine the presence of blood in a stain?
3. What methods are used to determine blood type?
4. By what systems is blood individualization checked?
5. What are the isosystems identified in the human body?
6. What are the approximate methods that allow you to determine the presence of blood in a stain?
7. What methods are used to determine the group affiliation of a blood stain in forensic examination?

8. How is the regional origin of blood determined?
9. What is the difference between the blood of an adult and a baby?
10. What methods are used to determine whether the blood in physical evidence belongs to a specific person?
11. What methods are used to determine whether blood in physical evidence belongs to a man or a woman?
12. How is it determined whether the blood found in the physical evidence belongs to an elderly person or an elderly person?
13. What are the methods of determining the period of formation of a blood stain?
14. What methods are used to determine the amount of blood shed as a result of an injury?
15. When and by whom was the simultaneous determination of agglutinogens and agglutinins in blood spots proposed?

### **PART III. Forensic examination of sperm**

Semen fluid is considered as material evidence in the investigation of sexual crimes such as touching the honor, unnatural satisfaction of sexual desire using force, promiscuity, forcing a woman to have sex.

Semen fluid appears due to the activity of a number of glands, the prostate gland, Litre's and Cooper's glands. The morphological composition of sperm fluid consists of spermatozoa, germ cells, leukocytes, etc. Sperm often contains enzymes and amino acids.

One of the first questions in the examination process is to determine whether sperm are really present in the stain. Approximate and reliable methods have been developed for this.

Presumptive methods of determining the presence of sperm in a physical evidence stain include microcrystalline reactions, potato juice reaction, and ultraviolet examinations.

***Microcrystalline reactions*** (Florence, Barberio reactions) are not of practical importance at the present time. Sperm stains on a fabric can usually be distinguished by their appearance. If it is difficult to find spots, they are examined using ultraviolet rays. When examined under ultraviolet light, spermatozoa emit light-colored fluorescence. However, this method is difficult to use when sperm stains are suspected on synthetic fabrics, as such fabrics themselves may exhibit similar fluorescence under UV light.

***Check with potato juice.*** If physical evidence with semen stains is contaminated or mixed with blood, then it is tested with potato juice. Potato juice causes agglutination of all types of erythrocytes. Sperm inhibits this property of potato juice and agglutination of erythrocytes does not occur. Therefore, the result of the reaction is considered positive if the strain prepared from the tested stain inhibits the agglutination of standard erythrocytes under the influence of potato juice.

Reliable methods of determining the presence of seminal fluid in physical evidence include a number of experiments.

***Method of morphological examination.*** The method of morphological examination is based on the detection of the presence of spermatozoa in spots on physical evidence. In order to achieve this goal, methods of finding spermatozoa in the object carrying the stain or separating spermatozoa from the stain and then identifying them are used.

If only one spermatozoon (head, body, and tail parts together) is found, the result is considered positive. The separate finding of the head, body, and tail parts of the spermatozoon is not taken into account. There are different variants of this method, in which the spermatozoa are examined either on the fabric itself (for example, on a gauze swab taken from the vagina) or separated (for example, with alcohol, by making a print) without separating it from the stain-preserving materials. Sometimes, the presence of spermatozoa is studied by the morphological method in a smear prepared on a glass slide from a sample taken from the subject.

***Determining the presence of sperm by A.K. Seropyan by the method of concentrated release.*** For the experiment, small cuts of 0.3 x 0.4 cm to 0.3 x 0.7 cm in size are made from spots suspected to contain sperm. The particles are placed in test tubes in the prescribed manner. The cut pieces are soaked in 10% alcohol. It is left at room temperature for 20-24 hours. The subject windows are marked as well as the test tubes. Pieces cut from each test tube using a closed-ended pipette are transferred to the center of the glass slide and the sample is poured. Leave at room temperature until dry. With the help of a preparation needle, the pieces of the glass are cleaned, a covering glass is closed over the traces left on the glass, and it is stained with a solution of 1% fuchsin in 1% hydrochloric acid. It can be seen under a microscope. If at least 1 whole spermatozoon is found in the preparation, the presence of sperm is considered to be confirmed. The presence of sperm heads (even if they are in large numbers) in the test spot cannot be the basis for the presence of sperm.

***Chromatographic inspection methods.*** Chromatographic (paper or thin-layer chromatographies) examination method for a number of sperm in the case of aspermia, azoospermia, i.e. in the absence of spermatozoa in the seminal fluid, the

spot is small in size, or the spot is contaminated with various substances based on the complex determination of specific substances, such as choline, spermine, phosphatase ore, it proves that the spot is formed from sperm fluid.

In 1974, professor J. J. Jalolov developed a method for the simultaneous detection of choline, spermine, and acid phosphatase using paper chromatography in cases of aspermia, azoospermia, and necrospermia in cases where spermatozoids were not detected in the sperm spot.

***Determination of the presence of sperm on paper by the method of chromatography.*** For experiments, 0.6x1 cm pieces of sperm-like spots and their control areas and pieces of a known sperm sample were placed on a glass slide or in the grooves of a tablet in a solution of 1% sodium phenolphthalein phosphate in acetate buffer. sprinkled with The sequence of placement of objects on the lower surface of the Petri dish is determined. Then the fragments are placed on the capillaries installed in the Petri dish and 37<sup>0</sup>C is left to dry for 1 hour. Chromatographic paper is prepared: lines are drawn on it with a graphite pencil at intervals of 1 cm, and a starting line is marked at a distance of 2 cm from the lower edge. Parallel cuts of 0.3 cm are made from both sides of this line, the distance between these pairs of cuts should be approximately 0.4 cm. Into the prepared incisions, pieces (threads) of a test stain, object carrier and a known sperm sample are placed. The test spot, object carrier and pieces (threads) from the known sperm sample are placed on the bottom of a glass container with a solvent. When the solvent reaches the upper edge of the paper, the paper is removed, the solvent level is marked with a regular (graphite) pencil, and it is dried in a fume cupboard at room temperature. The lower part of the dried chromatographic paper is treated with Dragendorf's reagent, and the upper part is treated with 0.1% alkaline sodium. After exposure, a yellow spermine spot is detected at the starting line of the chromatogram, and a purple choline spot is detected above it. After spraying with 0.1% alkaline sodium solution, a pink sour phosphatase stain is formed near the front (finish) line. If spermine, choline and sour phosphatase zones are detected in the chromatograms of the examined objects and a known sperm sample at the same

time, the reaction is considered positive if they are not stained in the paths of the object carrier particles. The specific Rf for choline, spermine and sour phosphatase zones in the test objects and known sperm should be at the same level.

***Emission-spectral inspection method.*** The emission-spectral method of determining the presence of sperm in the stain is based on determining whether the stain is sperm fluid based on the microelements found. An electrophorogram can be used to determine whether a stain on physical evidence is a seminal fluid stain, which can distinguish sperm from serum based on the presence and location of proteins.

***Determining the type of sperm.*** After determining the presence of sperm in the examined object, the forensic expert must determine its group membership, that is, to whom it belongs. In most cases, this task is not assigned to the expert's decision. In very rare cases, it may be necessary to determine the type of sperm.

If the presence of sperm is determined by the morphological method, the species can be determined by the shape and size of the spermatozoa. In cases where the presence of sperm is determined by other methods, its type is studied using precipitation reactions, similar to blood tests.

***Determining the group membership of sperm and whether it belongs to a specific person.*** Sperm group membership is determined by studying group factors, as in blood tests. Most often, the examination is carried out according to the ABO system. Determining the group of sperm in the stain consists of several methods, the most commonly used of which are agglutinin absorption, absorption-elution and mixed agglutination methods.

For this, antigens belonging to the ABO system are checked in the sperm spot, because by detecting them, it is determined which blood group the sperm spot is formed from the male seminal fluid. It should also be noted that the antigen belonging to this group is found in the sperm of a man, to which blood group he belongs. For example, if antigen A is found in a sperm spot, then it can be assumed that this spot was created by a man belonging to the second blood group. If the blood of the suspected man belongs to the third (B) or first (O) group, then it can

be concluded that this stain was not formed by the sperm of this man.

Another thing should be taken into account when determining the identity of the sperm stain found in the physical evidence. This is also a feature of "separability". The population of the earth is divided into two "separators" and "non-separators". One group of them (85%) isolates antigens belonging to the ABO system in various secretions (sperm, saliva, sweat, urine, bile, tear fluid, feces, mucus, etc.), that is, they these antigens are present in the composition and they are included in the group of "separators". The second group of individuals (15%) find themselves in various secretions (sperm, saliva, sweat, urine, bile, tear fluid, feces, mucus, etc.). The ABO system does not distinguish antigens belonging to the blood group, and they belongs to the "non-distinguishing" group.

This feature of the organism is of great importance in the practice of forensic medicine. For example, if antigen B is found in sperm stains found in physical evidence, then the suspect man's blood also belongs to group B, but if this antigen is not found in his saliva, then it is said that the sperm stain did not originate from this man's sperm.

Segregation can be determined by examining the Lewis, Gm systems in the blood. In particular, members of the Le (a- b+) group will be "separators". And the group Le (a+ b-) is characteristic of "non-separable". It is not possible to clearly determine the separability of Le (a-b-) group.

Antigens belonging to the ABO system are tested in saliva to find differentiation. Before starting the examination, the person being examined is asked to rinse his mouth with water, and then the saliva released from the oral cavity is collected in a test tube. The collected saliva is placed in a centrifuge and the liquid part is poured into gauze. The gauze is dried at room temperature and sent as a sample to the expert, it is determined that it contains antigens belonging to the ABO system.

In addition to antigens belonging to the ABO system, it is also desirable to find a group of phosphoglucomutase isoenzymes in order to determine to whom the sperm in the spot belongs. The amount of this enzyme is the same in blood and

sperm.

Based on the above investigations, the forensic expert concludes that the stain on the material evidence sent for examination is made of sperm fluid, identifies it as belonging to a group, and makes a conclusion that it belongs to a certain person (accused or suspect).

Also, in the crimes of indecent assault and lewdness, the antigens of the victims in the vaginal and rectal secretions are on the stain, and the group factor identified in the examination of the stain may belong to the victims. For this reason, in order to determine whether the sperm belongs to a specific person, blood and saliva samples of the suspect and the victim are submitted to the forensic examination, in addition to the materials obtained for the examination of the sperm. In the autopsy, blood and bile are taken for laboratory examination to determine the separation.

***Determination of A and B antigens in sperm stains using  $\alpha$ -A and  $\alpha$ -B isohemagglutinating serums by the method of quantitative absorption reaction.***  
***Determination of the level of "resolution".*** Test tubes for the experiment are initially determined depending on the number of objects to be tested: "K.ob. №...(b), Ob. №...(b)," "K. ob. №...(a), Ob. №...(a)". On a torsion balance, 25 mg of sperm spot and its control area are measured and transferred to designated test tubes. 0.15 ml of 1:32 titer  $\alpha$ -B serum is dropped into test tubes marked with "b". 0.15 ml of 1:32 titer  $\alpha$ -A serum is dropped into test tubes marked with "a". Test tube tripod +4-6<sup>0</sup>C is left in the refrigerator for 24 hours. Absorbed serum tubes are placed in a row on a stand. 7 pieces of agglutination tubes marked with "S, 2, 4, 8, 16, 32, 64" are placed opposite each test tube. 2 drops of absorbed extract are dropped into the test tube marked with "S". 2 drops of physiological solution are dripped into test tubes marked with "2", "4", "8", "16", "32", "64". 2 drops are taken from the absorbed serum tubes using a pipette and transferred to the test tube marked "2". Mix the liquid without foaming (mixing is carried out by withdrawing the liquid into a pipette and returning it to the test tube), transfer 2 drops to the test tube marked "4". The liquid is mixed without foaming, 2 drops are removed to the

next test tube, and so on. After mixing the test tube marked with "64", 2 drops are taken (poured). 1 drop of 1% Ba(III) test erythrocyte mixture is added to test tubes marked with "b". 1 drop of 1% test-erythrocyte mixture of Ab(II) group is added to test tubes marked with "a". Centrifuge and shake for 4 minutes. 1 drop from each test tube is transferred to the object glass and closed with a cover glass. It can be seen under a microscope. If compared to the absorbed serum of the object carrier, the absorbed serum of the sperm stain has decreased its titer by no less than 3-4 steps of absorption, then the antigen is detected in the tested stain. If compared to the absorbed serum of the object carrier, the sperm stain absorbed serum decreases its titer by no less than 5-6 steps of absorption, the level of "resolution" is determined.

## **Control questions**

1. What can sperm and its traces look like at the scene?
2. How are sperm stains found at the scene?
3. In what order are sperm traces taken for laboratory examination?
4. What issues are resolved in the examination of sperm spots?
5. What are the approximate methods of determining the presence of sperm in a stain on physical evidence?
6. What are the reliable methods of determining the presence of sperm in a stain on physical evidence?
7. What methods are used to determine the type of sperm?
8. What are the methods of determining sperm group membership?
9. How is it determined that the sperm belongs to a specific person?
10. What do you mean by "separator"?
11. What samples are taken for laboratory examination to determine the separation in autopsy?
12. How are the methods of chromatographic examination of the presence of sperm in the stain performed?
13. When and by whom was the method of proving that the stain was formed from sperm liquid based on the complex determination of choline, spermine, and phosphatase at the same time created?
14. What method is used to determine the presence of sperm when the spot is formed by a person without spermatozoa in the semen, if the spot is small, or if the spot is contaminated with various substances?
15. Why are samples taken from sex crime suspects?

#### **IV. Forensic examination of hair**

Forensic examination of hair is important in cases of murder, sexual crimes, personal injury, theft, and traffic accidents. As a result of hair examination, answers to many questions are collected and they can be evidence in solving the crime committed.

In many cases, investigative agencies are required to determine the exact identity of hair found at a crime scene or on a crime weapon or on someone's clothing. are interested in questions about whether the hair is pulled out as soon as it falls out, dyed, cut, curled, and with what substances it is contaminated.

At the scene of the accident, hair can be found on weapons of injury, on the exterior and interior of vehicles, on the victim's clothing, and on the hands (especially between the fingers). Also, hair belonging to the victim can be identified on the accused's body and clothes.

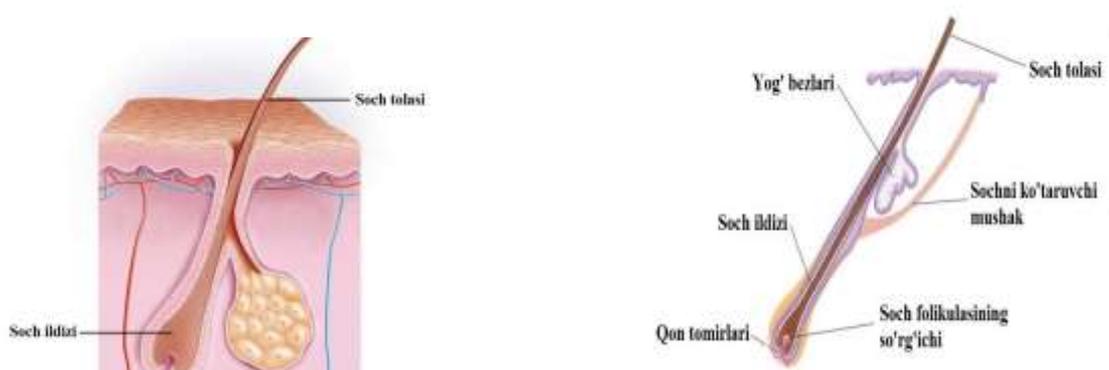
In good light at the scene, hair can be found with the naked eye or with a magnifying glass. Hair and all suspected objects are removed manually or with tweezers with a rubber handle in order to preserve the existing coatings and not to cause additional damage. Hairs taken from each area (body parts, bodies) are placed in separate paper bags or envelopes with appropriate marks and sent to the forensic laboratory.

It is advisable for a forensic expert to solve the following issues during hair examination:

- determine whether the object sent for inspection is hair;
- determining whether the hair belongs to a person or an animal;
- determining which area of the human body hair belongs to;
- hair group identification;
- determine which person the hair belongs to;
- determining what kind of object or weapon was used to injure the hair;
- determining whether there is a chemical or high temperature effect;
- determining whether the hair has fallen or been pulled out;
- determining whether the hair is plucked quickly or slowly;

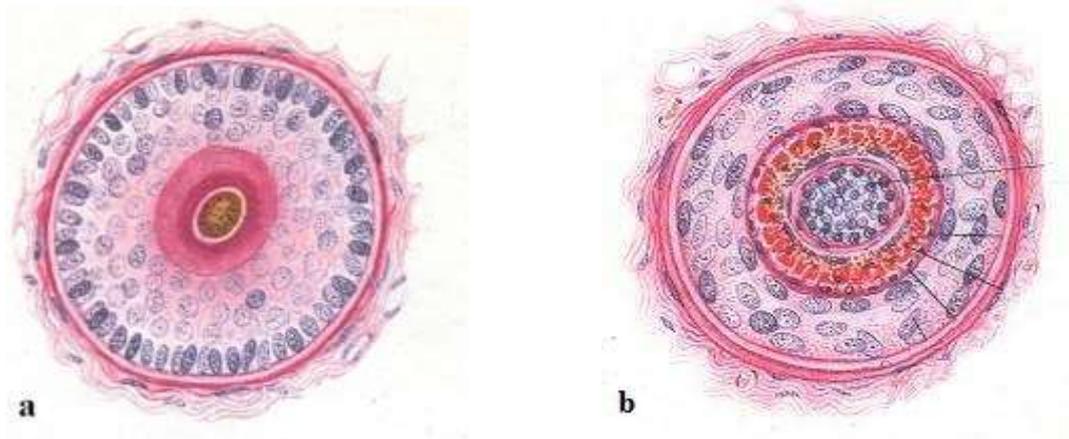
- to determine whether it is curled, painted or contaminated with any substance (firearms, etc.).

***Determining whether the object is hair or not.*** It is determined by microscopic examination that the object of examination is hair. Hair consists of a root inside the skin and an outer root. In the fiber part of the hair, there are different layers of cuticle, bark and core. The uppermost layer is called the cuticle. It is a flat tissue in the shape of a shingle. The second layer is called the hollow layer. The third layer is the core of the hair. The existence of these three layers proves that the object is undoubtedly hair.



**Figure 6. Morphological structure of hair.**

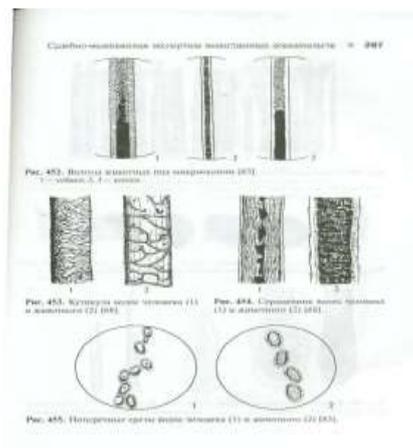
In some cases, it becomes impossible to determine the origin of the object under investigation, in such cases it is investigated using special methods. For this, the cuticle of the hair is examined, that is, the location of flat cells in the free part of the hair is studied. The location of the flat cells cannot be seen under a microscope, so the hair is imprinted on the emulsion surface of the photographic film. If flat cells are found in the examined object, this indicates that it is hair. For this, the cross-section of the examined object is studied, and importance is given to the shape of the nucleus and the location of the pigment. In addition, it is recommended to use the emission-spectral method when examining hair, and by studying its composition, it is determined whether the object being examined is hair or not hair.



**Figure 7. Daylong section of hair: a) from the lower third of the hair root; b) from the area of the hair follicle.**

***Determining whether the hair belongs to a person or an animal.*** Whether the hair belongs to a person or an animal is determined using a microscope. Human and animal hair differ in structure (Fig. 8).

The structure of the human hair shaft is indistinguishable; thickness of hair  $\frac{1}{3}$  does not exceed part; hair can be continuous in length, have the appearance of islands, and be continuous in the form of a strap. The thickness can vary depending on the length of the hair



**Figure 8. Hair cuticle: a - human, b - animal (according to I.A. Kontsevich (1988)).**

it won't happen. The structure of the core of animal hair is composed of several rows of cells of different shapes and sizes; thickness looks good on most hair,  $\frac{9}{10}$  can occupy a part up to; the length of the hair is usually in a continuous strap-like appearance, and only the tip or root may be long. Most of the time, the thickness is even, the core is also found in hair with a thickness of 0.011-0.012 mm.

In humans, the cortex makes up most of the hair; the pigment is located in the

same plane or close to the cuticle (may be centrally located in blonde-haired hair); the pigment grain can be small or medium-sized; pigmentophores can be small and medium. In an animal, the bark layer is a small part of the hair; pigment is centrally located (pigment may be located in a ring); pigment grains and pigmentophores are large.

The optical edge of the cuticle of human hair is flat, the teeth can be slightly distinguished, the cuticle cells are small and tightly packed; denticles converging or converging at different distances. The optical edge of the animal hair cuticle is uneven, the serrations are well visible, sometimes serrated and the serrations are distant.

The cuticle of human hair is complex, the lines are sometimes closer, sometimes farther apart, forming a zigzag shape; the serration of the free end of the cells is less bilinear, small, and the image is weakly expressed (Fig. 9).

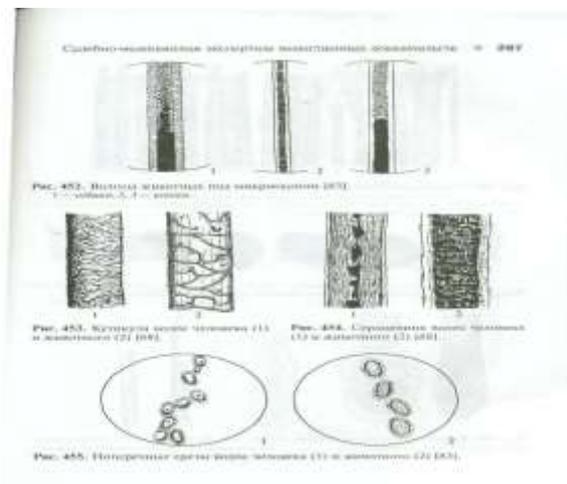


Figure 9. Structure of human (a) and animal (b) hair (according to I.A. Kontsevich (1988)).

Photo of animal hair cuticle simple, the lines are far from each other, sometimes it creates a unique appearance like a pine cone; lines large dentate; the waviness is weakly expressed and the cuticle pattern has changed along the length of the hair.

Depending on the location of the cells in the cuticle, the structure of the core, it is determined whether the hair of one animal is similar to the hair of another animal, which animal the hair belongs to.

***Determining which part of the body hair belongs to.*** For this, the length,

thickness, shape and cross-section of the hair are important. For example, the daily cut of the head hair is round or oval; mustaches and beards are triangular in shape; and the hair of the goat is kidney-shaped.

***Determining the identity of human hair found in physical evidence.*** To whom does the hair belong? It is determined by comparison of the hair presented as physical evidence and a sample (hair taken from the suspect). When determining who owns the hair, all signs are important: color, length, shape, thickness, appearance of the core, the structure of the root part and peripheral part, the presence of pigment, its color, location.

The expert makes a conclusion, taking into account whether the hair presented as physical evidence and samples are similar or not. An expert can judge the similarity of the hairs, but not exactly who they belong to, because hairs taken from different areas and heads of the same person are different from each other. In addition, different people's hair can be similar in texture. When drawing up his conclusion, the expert shows that the hair presented for examination as physical evidence is similar in structure to the hair of a certain person and thinks that it can be the hair of that person. If the hairs are not similar, this indicates that the hairs belong to different individuals.

When determining who the hair belongs to, an immunological test is performed to determine the hair's antigens belonging to the ABO system (the same antigens are found in the blood and hair of people). The results of the hair analysis are compared with the blood type of the suspects in the crime case and an opinion is made about who the hair belongs to.

***Determination of the ABO isoserological system group in hair by absorption-elution reaction method using  $\alpha$ -A,  $\alpha$ -B isohemagglutinating sera,  $\alpha$ -H elderberry extract.*** To conduct the experiment, the hair is first washed in soapy water, rinsed, and dried on a filter paper. The outer surface of the agglutination test tubes and the object glass are cleaned with ethyl alcohol. Hair is crushed using agglutination tubes on a microscope slide. Crushed hair is divided into 9 pieces, about 0.5-0.6 cm long. Tablet grooves are marked: b for serum; "ob. №...(b), A(b),

B(b), AB(b), O(b)". a for serum; "ob. №...(a), A(a), B(a), A(a), O(a)". A-H for elderberry extract "ob. No....(a-H), A(a-H), (a-H), A(a-H), O(a-N)". Crushed test hair and hair samples of a certain group are placed in the designated slots in 3 sets. 2 drops of b serum with a titer of not less than 1:128 are dripped into the grooves marked with b. 2 drops of a serum with a titer of not less than 1:128 are dripped into the grooves marked with a. 2 drops of a-H, elderberry extract with a titer of 1:64 are dripped into the grooves marked with α-H. Tablet +4-6<sup>0</sup>C is placed for absorption for 20 hours. Objects from serum wells are removed onto filter paper using tweezers. A deep-grooved tablet and a chilled physiological solution are placed in a special container with ice. For each subject, depending on the serum titer and the effect of the subject carrier, 3-5 rows of grooves with chilled saline <sup>2</sup>/<sub>3</sub> will be filled up to the part. Objects to be tested are placed in the first row of slots under the control of a 2-minute timer using tweezers. After 2 minutes, the object is transferred to the second groove using tweezers, etc. After washing, the objects are transferred to a clean filter paper and dried. A 1% albumin solution is prepared from AB(IV) blood serum (0.1 ml of AB(IV) blood serum and 9.9 ml of physiological solution are mixed). erythrocytes of Ab(II), Ba(III) and Oab(I) groups are washed: physiological solution is added to erythrocytes, centrifuged for 15 minutes, the liquid above is removed, physiological solution is added again and centrifuged for 15 minutes. Washing is repeated 3 times. After washing, all saline is removed. Preparation of a mixture of erythrocytes of groups Ab(II), Ba(III) and Oab(I) washed three times in 0.2-0.3% albumin solution (0.05 ml of undiluted erythrocytes are added to 10 ml of 1% albumin solution). The mixture of erythrocytes is checked for spontaneous agglutination. The slide is wiped with ethyl alcohol, they are marked like the grooves on the absorbed tablet, the slide is placed in a wet chamber. 2 drops of 0.2-0.3% Ba(III) group erythrocyte mixture on the object glasses marked with "Ob. No....(b), A(b), B(b), AB(b), O(b)" drips and the drops are absorbed by the serum, washed hair is passed through 3. 2 drops of 0.2-0.3% Ab(II) group erythrocyte mixture on the object glasses marked with "Ob. No....(a), A(a), B(a), AB(a), O(a)" drips and the drops are absorbed with a serum,

the washed hair is passed through 3. 2 drops of 0.01% Oab(I) group erythrocyte mixture are added to the glass slides marked with "Ob. No....(a-H), A(a-H), B(a-H), AB(a-H), O(a-H)" and a-H Washed hair absorbed with herbal elderberry extract is passed through 3. Wet chamber where the subject windows are placed to the thermostat +48-52<sup>0</sup>30 minutes are put on S. After removing the object from the thermostat, the moist chamber with glass is kept on the table for 2 hours. Examined under a microscope. If agglutination is observed in the glasses marked with "b", B antigen; If agglutination is observed in the windows of the subject marked with "a" antigen A; If agglutination is observed in the slides marked with " $\alpha$ -H", it indicates that H antigen has been detected. If agglutination is observed on glass slides containing hair samples of a certain group and erythrocytes corresponding to them (between  $\beta$  and B, AB and  $\alpha$  and A, AB as well as  $\alpha$ -H and O), the reaction is considered to have been carried out appropriately and the group belonging to the hair has been determined.

***Determining the similarity of hair.*** Various instrumental inspection methods are recommended for this purpose. These include: hair refraction, light transmission coefficient, gravimetric properties, calculation of the number of cuticle lines, measurement of cross-sectional area, study of breaking resistance, emission-spectral analysis of the composition of elements, macro- and microluminescence analysis, examination in polarized light, histochemical method, atomic absorption analysis, spectrophotometric examination using infrared rays and others.

***Determining the effect of external factors on hair.*** Hair exposed to high temperature will change its appearance, it will have a flowing appearance. Under a microscope, you can see gaps or burns in the hair fibers. If the hair is cut quickly, then the cut end will be straight. Gradually, the cut ends of the hair are stair-shaped. Recently cut hair has straight ends and sharp edges that smooth out over time (Figure 6). Sometimes there may be a question about whether the hair is curled, dyed or colored. To answer these questions, the hair is examined under a microscope. For example, curled hair can show signs of heat exposure.



**Figure 10. Different views of the peripheral end of human hair: supirgisimon; needle-thinned; cut with a sharp object; plucked with slow motion; fully polished; a tangled strand of hair.**

As a result of emission-spectral examination, it is possible to determine not only whether the hair is dyed, but also what kind of dye was used. As a result of this examination, an element can be found in large quantities in dyed hair, and in undyed hair, such an element can be either absent or in small quantities. This substance is found in large quantities in hair dye. Microscopic examination of hair suspected of gunshot wounds may reveal signs of burns, the presence of soot, and signs of injury from partially ignited gunpowder particles.

***Determining the gender of hair.*** Currently, it is possible to determine the gender of hair, which is determined using several methods. Sex chromatin is checked in the cells of the hair root, and by studying it, it is determined which sex the hair belongs to. In addition, in order to determine the gender of the hair, its chemical composition is studied. Men's and women's hair differ depending on the amount of some elements. The chemical composition of hair is determined by various methods. For this purpose, spectrophotometric tests are carried out using emission-spectral and infrared rays.

Hair differs from other human biological objects in that it withstands the process of decay for a long time. Hair color can change depending on the conditions (soil, air, water, swamp, temperature) where the corpse was left. For example, hair stored in soil can turn reddish-brown or reddish-yellow after several decades. They fade, lose their elasticity, but retain their structure. For this reason,

even when signs of injury disappear as a result of soft tissue decay, it is possible to identify them by examining the hair.

## **Control questions**

1. How is hair-like physical evidence found at a crime scene?
2. In what order are objects suspected of hair taken for laboratory examination?
3. What issues are resolved in the forensic examination of hair?
4. What methods are used to determine whether the subject is hair or not?
5. What is the difference between human and animal hair?
6. What methods of examination are used to determine the gender of hair?
7. For what purpose are hair samples taken from the suspect or accused?
8. How is it determined that the hair belongs to a specific person?
9. How is the regional affiliation of the hair determined?
10. How do you determine if your hair has been exposed to chemicals or high temperatures?
11. What changes are detected in the hair under the influence of impermeable objects?
12. What are the signs of a gunshot wound identified by a forensic examination of the hair?
13. What methods are used to determine the similarity of hair?
14. How is it determined that the hair was injured by an object or weapon?
15. How is it determined whether the hair falls out or is plucked out, and whether the hair is pulled out quickly or slowly?

## **V. Saliva, sweat, urine and other secretions of the human body forensic biological examination**

Saliva, sweat, urine, breast milk and Forensic biological examination of other parts of the human body is very important in solving the crime.

**Examination of saliva.** In most cases, the saliva test is carried out on the remains of cigarettes and cigarettes found during the inspection of the scene of the incident, on various items, on drinking vessels, on envelopes sealed with saliva.

If there is a suspicion of the presence of saliva on an item, the suspicious areas are first examined using ultraviolet rays. Under the influence of ultraviolet rays, saliva appears to be colored. As a reliable method to check the presence of saliva in physical evidence, a chemical experiment based on the detection of the presence of ptyalin is carried out. This method is based on testing by applying starch to the stain formed from the test spot. If the stain contains ptyalin, it breaks down the starch. Lugol's solution does not change the color of the decomposed starch. If the stain does not contain ptyalin, the starch does not break down. Lugol's solution reacts with undigested starch and produces a blue color.

After determining the presence of saliva in the stain, the expert determines to whom the saliva belongs personally by checking the group antigens of the saliva in these objects. The methods of agglutinin absorption, absorption-elution, and mixed agglutination methods used in blood group testing are used to determine the group belonging to antigens of saliva. In determining who the saliva belongs to personally, the characteristic of "separability" of the examinee, that is, the presence of antigens and agglutinins belonging to the ABO system in his saliva, is important.

***Determination of the presence of saliva by amylase enzyme.*** First, the centrifuge tubes for the experiment are placed on the rack and marked. The number of test tubes should correspond to the number of objects to be examined and the number of their subject carriers, as well as a test tube is placed for a specific saliva sample). The stain on the cloth suspected of saliva and pieces from the control area (object carrier) and the dried saliva sample on the gauze are crushed with scissors.

Particles should not exceed 100 mg (washes are taken from unbroken surfaces on screened gauze). Measurements are placed in test tubes as specified. The objects to be tested are poured with toluene so that they sink a little. Leave for 4 hours at room temperature. 5 ml of salt solution of potato starch is poured into each test tube. +37°C is left in the thermostat for 20-24 hours. Half of the liquid in each test tube is transferred to clean test tubes, 1 drop of Lugol's solution diluted 1:3 in distilled water is added. If the turbid solution becomes clear and Lugol's solution does not stain (that is, amylase breaks down all the starch into simple sugars), the presence of saliva is determined. If there is no or very little saliva in the test object, the solution will remain cloudy and turn blue when Lugol's solution is added. The appearance of a purple color in the liquid is not a reason to conclude about the presence or absence of saliva. If the amylase reaction gives a negative result (the solution turns blue) when testing particles of this weight, and a certain saliva sample gives a positive result (the solution looks colorless), the reaction is considered to have been duly conducted.

***Determination of A and B antigens in saliva stains using  $\alpha$ -A and  $\alpha$ -B isohemagglutinating sera by the method of quantitative absorption reaction. Determining the level of "separability".*** Test tubes for conducting experiments are determined depending on the number of objects to be tested: "K.ob. No....(b), Ob.No....(b)," "K. ob. №...(a), Ob. №...(a)". 25 mg of saliva stain and its carrier are measured on a torsion balance. Measurements are transferred to designated test tubes. 0.15 ml of 1:32 titer  $\alpha$ -B serum is dropped into test tubes marked with "b". 0.15 ml of 1:32 titer  $\alpha$ -A serum is dropped into test tubes marked with "a". Test tube tripod +4-6°C is left in the refrigerator for 24 hours. Absorbed serum tubes are placed in a row on a stand. 7 pieces of agglutination tubes marked with "S, 2, 4, 8, 16, 32, 64" are placed opposite each test tube. 2 drops of absorbed extract are dropped into the test tube marked with "S". 2 drops of physiological solution are added to test tubes marked with "4", "8", "16", "32", "64". 2 drops are taken from the absorbed serum tubes using a pipette and transferred to the test tube marked "2". Mix the liquid without foaming (mixing is carried out by withdrawing the

liquid into a pipette and returning it to the test tube), transfer 2 drops to the test tube marked "4". The liquid is mixed without foaming, 2 drops are removed to the next test tube, etc. After mixing the test tube marked with "64", 2 drops are taken (poured). 1 drop of 1% Ba(III) test erythrocyte mixture is added to test tubes marked with "b". 1 drop of 1% test-erythrocyte mixture of Ab(II) group is added to test tubes marked with "a". Centrifuge for 4 minutes, shake. 1 drop from each test tube is transferred to the object glass and closed with a cover glass. It can be seen under a microscope. If the titer of the absorbed serum of the saliva stain compared to the absorbed serum of the object carrier has decreased by no less than 3-4 steps of absorption, then the antigen is detected in the examined stain. If compared to the object-carrying absorbed serum, the absorbed serum of the saliva stain reduces its titer by no less than 5-6 absorbance levels, the level of "resolution" is determined.

**Sweat test.** Traces of sweat are also the object of forensic examination in most cases. The main composition of sweat is the secretion of sweat glands. 4 gr in 1 hour from the human body. sweat may be released. This indicator depends on the functional state of the body, thermal and mental effects, taking medications and other factors. Sweat is usually a colorless liquid that can become different colors under the influence of a number of microorganisms. When sweat is absorbed in large quantities in white tissues, it has a yellow color. Sweat marks may not be visible on fabrics dyed in different colors. However, in areas where sweat is constantly absorbed, the fabric becomes discolored or the color of the fabric changes permanently.

Sweat mainly consists of water (97-99%) and a number of other trace elements, including sodium, potassium, calcium, magnesium, proteins, fats, enzymes, amino acids. Among amino acids, serine content is more than others. A method based on the detection of serine amino acid has been developed to determine the presence of sweat on a stain. Serine is always present in the sweat and in a sufficiently high concentration and has a specific property. Serine is present in small amounts in other biological fluids and cannot be detected in normal chemical reactions. The amount of serine in sweat does not depend on

diseases associated with metabolic disorders, what kind of food was taken, and in which part of the body the sweat was formed. Serine is in the form of colorless crystals, resistant to normal temperatures and decomposes at 228 degrees. The method of determining the presence of serine in sweat is based on the fact that serine is oxidized under the influence of sodium periodate to form formaldehyde.

The presence of sweat is carried out by checking various clothes. Sections of the evidence are cut and tested for the presence of the amino acid serine. Finding sweat in physical evidence and determining its antigenic group affiliation can help investigative agencies prove who owned or wore the examined clothing. In some cases, the presence of sweat can be found on hair combs or clips found at the scene of the accident. This check also takes into account the "discretion" characteristic of a person.

***Determination of the presence of sweat by serine, threonine, valine and leucine amino acids by chromatography on silofol plate.*** For this, the agglutination test tubes are placed on the stand. Approximately 0.5-1 cm from objects, object carrier and known sweat samples<sup>2</sup> size pieces are cut. The particles are placed in test tubes in the prescribed manner. Physiological solution is dripped into the test tubes so that the particles sink (depending on the duration of the formation of the sweat spot, extraction with 5% acetic acid can also be carried out). Leave at room temperature for 24 hours for extraction. A silufol plate is prepared: with a scalpel or pointed tweezers, longitudinal lines are made at 1 cm intervals, the drawing is performed by removing the outer layer of the aluminum plate. A starting line is marked with a graphite pencil at a distance of 1.5 cm from the bottom edge. 20 object pulls using glass capillaries, object carrier and known sweat sample pulls are layered (pencil) to the starting line. The silofol plate, folded in layers, is placed in a chamber with a universal solvent at the bottom and closed with a lid. After the solvent reaches the upper edge of the plate (after about 3-4 hours), it is removed from the chamber and +100<sup>0</sup>C is placed in the thermostat until the smell of acetic acid disappears (about 30 minutes). The heated plate is treated with a 1% solution of ninhydrin in alcohol. +56 to the plate thermostat<sup>0</sup>It is

placed in C for 15-20 minutes. If after showing the chromatogram, the significant Rf at different levels of silufol plate: serine - 0.23; threonine - 0.33; valine - 0.4; If pink-purple spots corresponding to leucine - 0.53 amino acids are formed, the presence of sweat is determined. The results are recorded in the work log. If a significant pink-violet color Rf is formed in the way of weighing a specific sweat sample and is not formed in the way of weighing the object carrier, the reaction is considered to have been carried out appropriately.

***Determination of A and B antigens in sweat stains using  $\alpha$ -A and  $\alpha$ -B isohemagglutinating sera by the method of quantitative absorption reaction.***

First, test tubes are marked: "K. ob. №...(b), Ob. №...(b)," "K. ob. №...(a), Ob. №...(a)". 25 mg of sweat stain and its carrier are measured on a torsion balance. Measurements are transferred to designated test tubes. 0.15 ml of 1:32 titer  $\alpha$ -B serum is dropped into test tubes marked with "b". 0.15 ml of 1:32 titer  $\alpha$ -A serum is dropped into test tubes marked with "a". Test tube tripod +4-6<sup>0</sup>C is left in the refrigerator for 24 hours. The start time of reaction execution is recorded in the work log. Absorbed serum tubes are placed in a row on a stand. 7 pieces of agglutination tubes marked with "S, 2, 4, 8, 16, 32, 64" are placed opposite each test tube. 2 drops of absorbed extract are dropped into the test tube marked with "S". 2 drops of physiological solution are dripped into test tubes marked with "2", "4", "8", "16", "32", "64". 2 drops are taken from the absorbed serum tubes using a pipette and transferred to the test tube marked "2". Mix the liquid without foaming (mixing is carried out by withdrawing the liquid into a pipette and returning it to the test tube), transfer 2 drops to the test tube marked "4". The liquid is mixed without foaming, 2 drops are taken into the next test tube and Sh.k.. After mixing the test tube marked with "64", 2 drops are taken (poured). 1 drop of 1% Ba(III) test erythrocyte mixture is added to test tubes marked with "b". 1 drop of 1% Ab(II) test-erythrocyte mixture is added to test tubes marked with "a". Centrifuge for 4 minutes. Shaken. 1 drop from each test tube is transferred to the object glass and closed with a cover glass. It can be seen under a microscope. If the serum absorbed by the sweat stain has decreased its titer by no less than 3-4 absorbance

steps compared to the absorbed serum carrying the object, it is considered that the antigen has been detected in the tested stain.

**Urine test.** When a spot similar to a urine stain is found at the scene of the incident, a urine test is conducted. The presence of urine in the stain is determined using a chemical reaction, that is, by checking the presence of creatinine, which is the main part of urine. Urine also contains antigenic grouping, which helps to identify the identity of the urine. This also takes into account the "dissolving" property of urine.

The question of determining pregnancy and childbirth based on urine stains may arise in the investigation of infanticide, criminal abortion and other crimes. Several methods have been proposed to determine pregnancy and childbirth based on liquid urine and urine stains.

Among the biological tests, the Aschheim-Tsondek test, which is mainly used in the clinic, is based on the detection of the hormone prolactin, which is produced in the pituitary gland. This hormone is stored in the urine of pregnant women. When this hormone is injected into the body of sexually immature female white mice, it causes premature development of secondary sexual characteristics. However, this reaction is not widely used in the practice of forensic medicine due to the fact that it requires a lot of work and requires the use of animals.

One of the immunological tests is based on the detection of the presence of the hormone chorionic gonadotropin, which is developed in the placenta. This hormone is constantly present in the blood and urine of pregnant women and is stored throughout the period of pregnancy from 5-9 days. During 5-9 days of pregnancy, the concentration of chorionic hormone is 2500-100000 IU, in 6-12 and 30-36 weeks it is around 100000 IU. After childbirth or abortion, the amount of chorionic hormone disappears in the blood and urine.

***Determination of the presence of urine by the method of thin-layer chromatography on a silicofol plate.*** Marked agglutination test tubes are placed on the stand. The number of test tubes should correspond to the number of objects to be tested, and a test tube is also placed for a specific urine sample. About 0.5-1 cm

from the test objects and a known urine sample (washes are made from non-abrasive surfaces onto the tested gauze)<sup>2</sup> size pieces are cut. The particles are placed in test tubes in the prescribed manner. The pieces in the test tubes are dripped with physiological solution (or distilled water). Leave at room temperature for 24 hours for extraction. A silufol plate is prepared: with a scalpel or pointed tweezers, longitudinal lines are made at 1 cm intervals, the drawing is performed by removing the outer layer of the aluminum plate. A starting line is marked with a graphite pencil at a distance of 1.5 cm from the bottom edge. To the starting line, the weight of the objects to be examined using glass capillaries (1-2 times) and the weight of the known sweat sample (with a pencil) are layered in the prescribed manner. The silufol plate, which is layered with starches, is placed in a glass chamber with a universal solvent at the bottom and closed with a lid. After the solvent reaches the upper edge of the plate (after about 3-4 hours), it is removed from the chamber and +100°C is placed in the thermostat until the smell of acetic acid disappears (about 20 minutes). The heated plate is treated with 1% paradimethylaminobenzaldehyde. If a yellow spot with Rf=0.46 is formed in one-third of the tracks on the silufol plate after treatment with the chromatogram developer, the presence of urine is determined. If a yellow spot with Rf=0.46 is formed on the layered path of the concentration of a certain urine spot, the reaction is considered to have been carried out appropriately.

***Determination of the ABO isoserological system group in urine stains by absorption-elution method using  $\alpha$ -A,  $\alpha$ -B isohemagglutinating sera, as well as  $\alpha$ -N elderberry extract.*** Urine stain to be tested for the experiment, its object carrier, 0.2x0.2 cm from urine samples of known groups Oab(Í), Ab(ÍÍ), Ba(ÍÍÍ), AB(ÍV)<sup>2</sup> sized pieces or Separate strands 0.5-0.6 cm long are cut. Agglutination tubes are marked. For example: for b isohemagglutinating serum "K ob. No....(b), ob. No....(b), KD(b), A(b), B(b), AB(b), O( b)"? for isohemagglutination serum "K ob. No....(a), ob. No....(a), KM(a), A(a), B(a), AB(a), O(a) "; "K ob. No....(a-H), ob. No....(a-H), KD(a-N), A(a-H), B(a-H), AB(a-H), O (a-H)". Each object is divided into 3 parts, and each piece (or 3 strands of 0.5-0.6 cm long) is placed in

test tubes as indicated. 2 drops of b isohemagglutinating serum with a titer of 1:128 are added to the series of test tubes marked with "b". 2 drops of a isohemagglutinating serum with a titer of 1:128 are added to the series of test tubes marked "a". 2 drops of elderberry (a-H) extract with a titer of 1:64 are added to the series of test tubes marked with " $\alpha$ -H". Test tube tripod +4-6<sup>0</sup>C is placed in the refrigerator for absorption for 20-24 hours. Objects from serum tubes are removed onto filter paper with the help of closed-ended pipettes. A deep-grooved tablet and a chilled physiological solution are placed in a special container with ice. For each subject, depending on the serum titer and the effect of the subject carrier, 3-5 rows of grooves with chilled saline  $\frac{2}{3}$  will be filled up to the part. The objects to be examined are taken using thin-tipped tweezers and placed in the first row of grooves under the control of a 2-minute timer. After 2 minutes, the object is transferred to the second groove using tweezers, etc. After washing, the objects are transferred to a clean filter paper and dried. Clean tubes are labeled as above and dried objects are transferred to clean tubes using tweezers. 2 drops of physiological solution are dripped into each test tube. Test tube tripod +56<sup>0</sup>C is placed in the thermostat for 30 minutes. Threads (particles) in test tubes are removed on a clean filter paper using a pipette. 1 drop of 1% test-erythrocyte mixture of Ba(III) group is dropped into test tubes marked with "b", 1 drop of 1% test erythrocytes of Ab(II) group is dropped into test tubes marked with "a", 1 drop of Oab is added into test tubes marked with "a-H". A 1% test-erythrocyte mixture of group (I) is dripped. It is centrifuged and shaken for 4 minutes at 1500 rpm. 1 drop from each test tube is transferred to the object glass, a cover glass is closed on it and viewed under a microscope. In cases where agglutination is observed in the test tubes of object carriers, it is necessary to carry out measures to eliminate the effect of object carriers. If agglutination is not observed in the test tubes of test objects and their object carrier weights, the reabsorption-elution reaction should be carried out starting from the absorption phase. The absorption phase is carried out several times: threads of objects are poured with serums for 2 hours, serums are removed using a pipette, again poured with serums for another 2 hours. After this

manipulation is carried out several times, a complete absorption reaction is carried out with the last portion of the serum for 20-24 hours. If agglutination is observed in the series of test tubes marked with "b", it indicates that antigen B has been detected; If agglutination is observed in the series of test tubes marked with "a", it indicates that antigen A has been detected; If agglutination is observed in the series of test tubes marked with " $\alpha$ -H", it indicates that the H antigen has been detected.

**Examination of the skin and tissues.** In the practice of forensic medicine, in many cases, crime weapons can be found at the scene of the incident with traces of skin, some soft tissues, and their cellular elements. They should be subjected to forensic examination as material evidence in the investigation of the crime. In the process of forensic examination, by determining whether these objects really belong to a person, their gender and group affiliation, they can be used as evidence in the opening of a criminal case.

Gender is determined by cell elements, blood stains, and hair preserved in the objects that caused the injury. Such examinations should be carried out by a specially trained specialist who knows cytological examination methods. Identification of cell elements, organ-tissue characteristics, and genetic gender are determined in the tests. The objects are then given to biologists to determine their species and group. The physical evidence is first examined visually, then examined using a stereomicroscope. Pieces of tissue found in items and weapons are placed in precipitation test tubes, and a small amount of isotonic solution is poured over them. The dough is made in the refrigerator for 3-72 hours. Then the pieces are crushed with a needle until a uniform substance is formed. If there is a small amount of cells on the surface of the arms, it is removed from this area using a clean gauze soaked in an isotonic solution. The sample taken from the object is placed in a test tube and a small amount of isotonic solution is placed on it. The tart is prepared in the refrigerator for 1 day. After this time, gauze is removed from the test tube in a compressed state. The resulting precipitate is placed on a glass slide and examined under a microscope. The sex of objects is assessed by checking the presence of X and Y chromatin. The examination begins with the identification

of Y chromatin, because it allows to determine not only the sex of the object, but also the species. The presence of Y chromatin in a single cell indicates that the object belongs to a person. Y-chromatin is found in the nucleus of male cells when treated with acridine and similar preparations. X-chromatin is determined by fluorescence microscopy. It is not difficult to make a conclusion about sex if there are enough cells that are suitable for making a diagnosis. If there is a small amount of cells suitable for diagnosis, the Wald method is used.

**Examination of organs and tissues.** A'zolar and tissues are relatively rare objects of forensic medicine expertise. In this case, the following questions are solved: the members of the inspected object and relatedness to tissues, species dependence, specificity of groups, sex, determining the relevance of organs and tissues to pregnant women is given special importance.

The object of the examination is considered to be the dismembered parts of the corpse or the large remains of the plane crash, and there is no doubt that they are related to a person. In such cases, the purpose of the examination is to determine whether the individual pieces found can be made from the same corpse or whether the found remains belong to a specific person.

For this, it is necessary to determine the characteristics of the objects of expertise by sex and groups, and also to study whether they originate from a pregnant woman. Small pieces of tissue, cell coverings in traumatic objects, organs and tissues can be submitted for examination. However, their origin cannot be determined without special investigation. In such cases, the expert is faced with all or almost all of the indicated questions. Sexuality of internal organs and tissues and cell elements are checked by cytological method. The rest of the members will undergo serological examination.

***Determine the relationship of organs and tissues to species.*** For this purpose, the examined objects (soft tissues with scissors, and bones with a saw) are crushed and separated in an isotonic solution of table salt. When the material is in sufficient quantity, separation is checked by precipitation reaction. If the size of the object is limited, it is better to use a more sensitive alternative method of immuno-

electrophoresis.

***Determining group membership of organs and tissues.*** Antigens of the ABO isoserological system were first discovered in human organs and tissues in 1927. Later it was found that group antigens are present in almost all organs and tissues. The information of different researchers about the discovery of antigens of other systems is contradictory. However, it is possible to determine many isoserological and serum group factors in sections of non-bleached organs and tissues.

At present, antigens of the ABO system are mainly detected in organs and tissues. Quantitative absorption reaction is used for this purpose. However, since it was not possible to detect weak antigens, accurate results were not always obtained. A more sensitive reaction is the absorption-elution reaction.

***Determination of the group of the ABO isoserological system in fragments of muscles, bones and organs by the absorption-elution method with the help of a and b isozerums,  $\alpha$ -H (herbaceous elderberry extract).*** To conduct the experiment, pieces of 0.2x0.2 cm in size are cut from the tested muscle and muscle samples of a certain group. The examined bone is reduced to a scaly form with the help of an ego, attached to an adhesive tape, and pieces of scaly adhesive tape measuring 0.4x0.4 cm or individual bone fragments with an average size of 0.2x0.2 cm are reacted. . Tablet grooves are marked: "ob. No. ..., A, B, AB, O". Objects to be tested and samples of certain groups are placed in the grooves as specified, and ethyl alcohol (with methyl and butyl alcohol - fixation time is 20 minutes) is poured over them for 1 hour. Objects to be tested are taken out on filter paper and dried. Agglutination test tubes are marked: b isohemagglutinating serum "ob. No....(b), A(b), B(b), AB(b), O(b)"? for isohemagglutinating serum "ob. No....(a), A(a), B(a), AB(a), O(a)"? For  $\alpha$ -H (herbaceous elderberry) extract "ob. No....( $\alpha$ -H), A( $\alpha$ -H), B( $\alpha$ -H), AB( $\alpha$ -H), O( $\alpha$ -H)". Each fixed object is divided into 3 parts, and each part is placed in test tubes in the specified manner. 2 drops of physiological solution are dripped into all test tubes with muscle pieces and the thermostat is set to +56<sup>0</sup>C is placed for 30 minutes. After 30 minutes, the

fragments are removed from the test tubes and transferred to clean, appropriately labeled test tubes. 1% erythrocytes of Ba(III) group are added to test tubes with physiological solution from which muscle pieces have been removed: 1 drop marked with "b"; 1 drop of 1% erythrocytes of Ab(II) group is added to marked "a"; 1 drop of 1% erythrocytes of Oab(I) group is added to marked "a-H". The test tubes are centrifuged at 1500 rpm for 4 minutes. Shaken. 1 drop from each test tube is transferred to the object glass, a cover glass is closed on it and it is seen under a microscope. Absence of agglutination indicates that fixation is sufficient. 2 drops of b isohemagglutinating serum with a titer of 1:128 are added to the series of test tubes marked with "b". 2 drops of a isohemagglutinating serum with a titer of 1:128 are added to the series of test tubes marked "a". 2 drops of elderberry (a-H) extract with a titer of 1:64 are added to the series of test tubes marked with "a-H". Test tube tripod +4-6°C is placed in the refrigerator for absorption for 20-24 hours. Objects from serum tubes are removed onto filter paper using a pipette. A deep-grooved tablet and a chilled physiological solution are poured into a special container with ice. For each subject, depending on the serum titer, 3-5 rows of wells are filled with chilled saline.  $\frac{2}{3}$  part is filled out. The objects to be examined are taken using thin-tipped tweezers and placed in the first row of grooves under the control of a 2-minute timer. After 2 minutes, the object is transferred to the second slot using tweezers. After washing, the objects are removed on a clean filter paper and dried. Clean tubes are labeled as absorbance tubes and dried objects are placed in them with tweezers. 2 drops of physiological solution are dripped into each test tube. Test tube tripod +56°C is placed in the thermostat for 30 minutes. Particles in test tubes are removed on filter paper using closed-ended pipettes. 1 drop of 1% erythrocytes of the Ba(III) group is added to the test tubes marked "b", 1 drop of 1% erythrocytes of the Ab(II) group is added to the test tubes marked "a", 1 drop of 1% erythrocytes of the Oab(I) group is added to the test tubes marked "a-H". . Test tubes are centrifuged at 1500 rpm for 4 minutes. Shaken. 1 drop from each test tube is transferred to the object glass, a cover glass is closed on it and viewed under a microscope. If agglutination is not observed in test tubes, re-

absorption-elution reaction should be carried out starting from the absorption phase. The absorption phase is carried out several times (sequential absorption): threads of objects are placed with serums for 2 hours, serums are removed using a pipette, again placed with serums for another 2 hours. After this manipulation is carried out several times, a complete absorption reaction is carried out with the last portion of the serum for 20-24 hours. If agglutination is observed in the series of test tubes marked with "b", it indicates the detection of antigen B, if agglutination is observed in the series of test tubes marked with "a", it indicates the detection of antigen A. If agglutination is observed in the series of test tubes marked with " $\alpha$ -H", it indicates that the H antigen has been detected. If a negative result is obtained after all the manipulations, it will not be possible to tell about the group affiliation of muscles and bones. If agglutination with compatible erythrocytes is observed in test tubes containing pieces of muscle, bone or organs of a certain group (between  $\beta$  and B, AB and  $\alpha$  and A, AB as well as between  $\alpha$ -H and O), the reaction has been carried out appropriately and is being tested. group membership of objects is determined.

There are many difficulties in determining the relationship of decayed tissues to groups. In this case, weakening or destruction of antigens is observed, and a non-specific risk is caused by microflora. The mixed agglutinin reaction provides good results in the detection of ABH group antigens in histological and cytological preparations, as well as cell elements. Attempts are being made to use the immunofluorescence method to determine the relationship of separated cells to groups.

## **Control questions**

1. What can traces of saliva, sweat and urine look like in physical evidence?
2. How are saliva, sweat and urine stains found at the scene?
3. How are traces of saliva, sweat and urine taken for laboratory examination?
4. What issues are resolved in the forensic examination of saliva stains?
5. What issues are resolved in the forensic examination of sweat stains?
6. What issues are resolved in the forensic examination of urine and its traces?
7. What are the ways to determine pregnancy and childbirth based on urine stains?
8. Skin and tissues What issues are resolved in forensic examination?
9. Organs and tissues What issues are resolved in forensic examination?
10. What methods are used to determine the relationship of organs and tissues to species?
11. What methods are used to determine the relationship of organs and tissues to groups?
12. Why is a saliva sample taken from suspects in sex crimes?
13. How is it determined that saliva belongs to a specific person?
14. What is the basis for determining the presence of saliva, sweat and other secretions?
15. What tests are used to determine whether the dismembered parts of the corpse belong to the same person?

## VI. Forensic cytology

**General features of forensic cytological examination.** Some of the cytological examinations have been used for a long time in the practice of forensic medical examination of physical evidence. When cellular elements are found in traces of blood, it is necessary to solve the problem of determining where they came from. Since ancient times, the presence of sperm has been determined by the finding of spermatozoa. The systematic use of cytological examination in the examination of physical evidence has made it possible to determine their sexuality based on the sex chromatin in the cell nucleus only recently. Therefore, the role of the cytological method in forensic medicine has increased.

Questions to be resolved in forensic cytological examination:

- to determine the sex of the blood on the stain;
- detection of saliva on stains, mainly on cigarette and cigarette residues, postal envelopes and stamps;
- find the elements of the root sheath of the cut hair;
- examination of the corpse for dismembered organs and tissues for small fragments and organ and tissue particles;
- finding cell elements in the instruments that caused the injury;
- finding particles of cell elements or specific organs and tissues related to animals in various objects, especially in traumatic instruments;
- to determine the presence of vaginal cells in sperm and blood stains on the clothes of persons suspected of sexual crimes, as well as by taking marks from the genitals and fingernails of suspected persons;
- finding spermatozoa to determine the presence of sperm in the stain.

Placental teats to determine the sex of the baby when a woman in labor comes to the hospital with an intact placenta, and the cells of the mucous membrane of the mouth of a person with anomalous sexual development can be the objects of a forensic cytological examination to determine the genetic sex.

Usually, the method of cytological analysis of physical evidence is carried out after the forensic biological examination. However, after the microparticles in

the instruments are taken by an expert cytologist, they are rationally distributed for examination in cytological and serological methods.

***Determining the relevance of the object of examination to organs and tissues.*** The need to carry out such an examination, when determining the presence of particles of organs and tissues in the wounding instrument and other objects, investigative agencies set a number of tasks before the forensic cytological expert. In such cases, first of all, it is necessary to determine the nature of the particles, i.e., whether they are from an animal (human) organism or some kind of plant, or whether they are not biological in nature. Particles are separated from the object using a stereomicroscope, and then preparations are made from them. Cytological examination easily determines the cellular structure of biological particles and makes it possible to compare whether they are of animal or plant origin. If it is unknown whether the particle is related to the animal, the serological method (precipitation reaction) is used.

When the object of examination is very large, its relation to the organ or tissue is determined by histological examination. Dried tissue pieces are stored in a wet chamber or in a special liquid, because under these conditions they return to their original state.

***Finding cell elements related to the animal in the wound instrument and their comprehensive examination.*** In expert practice, it is often necessary to examine not only particles of organs and tissues, but also microparticles and even isolated cells. Such a test method was developed and recommended by A.P. Zagryadskaya and others (1982) in recent years. Under the supervision of a stereomicroscope, the coatings on the surface of the object are scraped (washed-scraped) after washing several times with a small amount of water. If biological coatings are not well visible using a stereomicroscope, then the surface of the object is wiped with a piece of wet gauze, and cell elements are separated from it, and cytological preparations are prepared. The dependence of the animal-related cells found in the drug on tissues is studied. For the separation of cell elements, the wash obtained is used to determine the presence of blood by chromatography and

to study the relationship of proteins to species using the precipitation reaction. Mixed agglutination reaction detects ABH group antigens in cells. After that, the sex of the cells is determined by the cytological method.

Methods of complex testing of cell elements have been developed in the course. This can be done on the edges of flat broken glass and sharp tools.

***Determination of the origin of epithelial cells.*** It is very important to check the vaginal cells, sperm, male genitalia, and things under the fingernails in the blood spot. N.G. Shalaev (1965) and S.I. Lyubinsky (1969) are among the scientists who founded such investigations. In the process of learning, it is necessary to compare the cells of different types of multi-layered flat epithelium. Epithelial cells of the skin epidermis, oral cavity, vagina, male urethra are of particular importance. Diagnosis of epithelial cells of the skin is first determined in the preparation of a layer, a small group or isolated flat non-branching epithelial cells. Epidermal cells with multifaceted, well-defined boundaries are often deformed, wrinkled, with edges and folds, and differ from vaginal epithelial cells with unclear boundaries, 26-56  $\mu\text{m}$  in length. An important differential diagnostic feature is the presence of X-chromatin in the nucleus, as its presence indicates that it is female. However, this does not solve the issue of whether it is formed from vaginal cells. Such a conclusion can be reached only after finding a large amount of glycogen in the cytoplasm, because the vagina of healthy mature women has a lot of glycogen. Epithelia that have migrated from other places (for example, the oral cavity) do not contain glycogen. The finding of only one glycogen does not prove that the cell is of vaginal origin unless the sex chromatin is detected, because glycogen may also be present in the mucosa of the male urethra. Glycogen and sex chromatin are determined in one preparation. The drug is first stained by iodine vapor and then counted against viable cells to check the number of glycogen-storing cells. It is noted that the amount of glycogen in the cells is 10% and higher. Then the preparations are frozen in methanol and stained with azur-eosin. If X-chromatin is not found, the previously unstained part of the preparation is processed with fluorochrome to find Y-chromatin. An additional diagnostic factor

is the finding of a characteristic microflora bacillus on the cell surface. Determining the presence of sperm by the cytological method is not widely used in practice, but some forensic cytologists believe that spermatozoa can be accurately compared only by the head, its size, shape and specific structure.

### **Control questions**

1. What questions are resolved in forensic cytological examination?
2. How to determine the relationship of the object of examination to organs and tissues?
3. What experiments are carried out to determine the place of origin of epithelial cells?
4. How are the cell elements associated with the animal identified in the wound instrument?
5. In what order is the comprehensive examination of the cell elements related to the animal in the wounding instrument?
6. Diagnosis of vaginal epithelial cells in physical evidence?
7. What is the basis of the experiment to find the cells of the vulva in the root of the severed hair?
8. What are the methods of examining dismembered organs and tissues for small fragments and organ and tissue particles?
9. What is the experience based on the finding of cellular elements in mechanical instruments that cause injury?
10. What are the methods of checking the identity of the saliva in cigarettes and cigarettes?

## VII. DNA tests

**Structure and properties of DNA.** In 1869, F. Misher isolated a special substance with acidic properties in the cell nucleus and called it *Nuclein* (nucleus - nucleic acid) called In 1879, A. Kossel began to study the chemical composition of the nucleus. In 1889, R. Altmann introduced the term "nucleic acid" into the science and showed that nucleic acid contains nitrogenous bases, purine, pyrimidine, and 5-atom hydrogen (sugar). By the 1930s, the role of carbohydrates with five carbon atoms in the structure of nucleic acid and the fact that a group of carbohydrates in nucleic acid contained one less oxygen atom was discovered. P. Levin is a carbohydrate belonging to this group *dezoksiriboza*, and the other *riboza* called After that, nucleic acids were called deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). In a ribose molecule, an OH group is attached to a carbon, and an H atom is attached to a deoxyribose molecule.

DNA and RNA also differ in their nitrogen bases. in the DNA molecule *of nitrogenous bases* adenine, guanine, cytosine and thymine. In the RNA molecule, thymine is replaced by uracil. Adenine and guanine are purines, cytosine and thymine *pyrimidine bases* held as A combination of a nitrogenous base and ribose or deoxyribose *nucleoside* is called When a phosphoric acid residue joins a nucleoside, *nucleotide* is formed. Nucleotides are joined together by phosphoric acid to form a long strand of DNA or RNA. Nucleic acids are high molecular compounds, which include many nucleotides. A DNA molecule consists of 10-25 thousand nucleotides and has a high molecular weight.

In 1950, E. Chorgaff found that the number of adenine in the DNA molecule of all organisms always corresponds to that of thymine, and that of guanine always corresponds to the number of cytosine. Therefore, the number of purine bases and pyrimidine bases is equal. In all organisms, the nucleotides in the DNA molecule are similar, but they differ in number and order. Determining how the DNA molecule is structured has been a problem for many years.

In the 1950s, M. Wilkins, after calculating the results of X-ray examination of the DNA molecule by complex mathematical methods, obtained a spatial X-ray

image of the DNA molecule. Later, in 1953, the American geneticist J. Watson and the English geneticist F. Crick summarized their knowledge of DNA obtained by chemical and mathematical methods using X-rays and created a drawing (model) that clearly shows its structure. This drawing became known as Watson's Creek. According to it, the DNA molecule consists of two long and thin strands, which are intertwined and twisted around one axis, and are placed in a spiral. The length of a DNA molecule in a bacterial cell is 1 cm, and the length of a human body cell DNA molecule is more than 1 m.

Each strand that makes up the DNA chain is a polymer, one nucleotide in it is connected to another nucleotide through a phosphorus bond with deoxyribose. Both strands are connected to each other through nitrogenous bases. Adenine pairs with thymine (A-T) and guanine with cytosine (G-S). There are two hydrogen bonds between A and T, and three hydrogen bonds between G and Ts. It can be seen that G-S bases are more strongly interconnected than A-T. The distance between nucleotides is 34. The DNA strand forms a right-handed helix. One complete circle of it consists of ten nucleotides and its length is 34.

The DNA strand forms a right-handed helix. Its one complete circle consists of ten nucleotides and is 34 in length. The diameter of the double chain is equal to 20, because purine bases, whose ring length is equal to 12, combine with pyrimidine bases, whose ring length is 8.

In 1957, G.Sten showed that the double-stranded DNA molecule (replication) proceeds in the following 3 ways: 1) the double strand splits into two (semi-conservative), the double-stranded DNA molecule separates from each other without interruption, and each in front of one, a DNA chain that is complementary to them is formed; 2) a new DNA molecule is formed without breaking the double chain (conservative); 3) the double chain is broken and separated into pieces (dispersion), a new DNA chain is formed from the different structure of the dyes that arose from the breakdown of the original DNA molecule.

Half of the above methods explain the replication of the DNA molecule *conservative usul* It corresponds to the structure of the DNA structure proposed by

Watson and Crick. In the semi-conservative doubling of the DNA molecule, the hydrogen bond between the nitrogenous bases is first broken. After a break, the double chain begins to separate, and each crumpled chain takes the complementary nucleotides from the karyoplasm around it and forms a new chain. The DNA molecule created in this way is exactly the same as the previous one. A semiconservative method of DNA replication has been well studied in highly structured animals and plants.

A. Korenberg (1956) first studied the role of enzymes in DNA replication and from a bacterial cell *DNA polymerase* isolated the enzyme. Several other enzymes are involved in the duplication of DNA, not only DNA polymerase. For example, a chain of a DNA molecule is spread using the enzyme helicase. This enzyme acts against DNA strand twisting at high speed. In the bacterial chromosome, its rotation speed is equal to 4800 turns of DNA per minute. Double-strand breaks begin in single strands of DNA that are broken by an enzyme. A double strand of DNA is formed from the formation of a new bond opposite each new bond.

The enzyme DNA polymerase ensures the growth of a new DNA bond, which is formed from the 5' to the 3' side of a single-stranded DNA molecule. When a DNA molecule is broken, 5' and 3' fragments appear in it. The 5' fragment is the copy donor (matrix) in the synthesis of a new DNA chain. In the 3' dye, the necessary nucleotides for a new strand of DNA are collected, and a new strand of DNA grows and forms at the expense of this fragment. Therefore, the DNA fragment that forms the fragment with the last 3 numbers (zatravka) or *primer* is called All DNA polymerase enzymes initiate DNA synthesis from a site with a 3' - OH radical. Because the OH radical can quickly give way and a phosphate bond is attached to it. Therefore, synthesis will never be directed in the same direction at the same time on both strands of the double-stranded DNA.

According to R. Okazaki (1968), a new DNA molecule synthesized in each single strand of the DNA chain split into two is due to the formation of short polynucleotide pieces (fragments). The formation of a new DNA strand from these

primers is directed from the 5' primer to the 3' primer (5'→3'). These fragments are then joined together to form a common polynucleotide strand. A single, long strand synthesized from a 5'→3' fragment is called a dominant strand, and a strand formed from a 3'→5' fragment is called a lagging strand.

DNA polymerase synthesizes DNA fragments consisting of 100-200 nucleotides in eukaryotic cells, and 1000-2000 nucleotides in prokaryotic cells. When these fragments are joined together with the help of ligase enzyme, a common thread is formed.

DNA synthesis is a very fast process, in bacteria, 500 nucleotides are added to the mother strand (matrix) that creates a new strand every second, and in viruses, 900 nucleotides are added. All the DNA of my bacteria is completely regenerated within 20 minutes. In eukaryotic cells, this process is much slower. But there are many replicons in the chromosome of eukaryotic cells. Therefore, DNA synthesis starts at several places of one chromosome at the same time. A replicon is where DNA synthesis begins. A bacterial chromosome has only one replicon.

***Genetic code.*** How does a gene determine the structure (structure) of a protein whose amino acids have a certain order? How is the genetic information in the DNA molecule transferred to the protein, how many nitrogenous bases correspond to one amino acid?

Answers to these questions began to be collected since the 1960s. How the genetic information in the DNA molecule is transferred to the protein molecule is one of the biggest problems in the study of heredity. Although the protein molecule is complex, it consists of 20 monomers, that is, amino acids, but amino acids are combined in different numbers and in different order in the protein molecule. Therefore, there are many types of proteins. Twenty amino acids can meet (form a combination) in 104 different positions.

It is known that every difference between organisms is revealed through the difference in protein. A change of even one amino acid leads to a change in the structure of the protein. For example, substitution of valine for glutamine amino

acid in the hemoglobin protein causes severe anemia. In such patients, erythrocytes have a half-moon shape and have lost their charge. Therefore, the erythrocyte cannot attach oxygen to itself, and as a result, the patient cannot live long.

How does DNA determine protein diversity?

DNA is also a polymeric substance similar to protein. Protein consists of 20 monomers, while DNA consists of only 3 monomers (nitrogenous base, deoxyribose, phosphoric acid). All nucleotides have the same deoxyribose and phosphoric acid. The difference is only in nitrogen bases.

The difference between DNA molecules depends on the arrangement of nitrogenous bases in the DNA chain. The arrangement of nitrogenous bases determines the arrangement of amino acids in the protein molecule. So, the individual differences of organisms depend on the order of nitrogenous bases in the DNA molecule. The sequential arrangement of nitrogenous bases in the DNA molecule, which determines the arrangement of amino acids in the synthesized protein molecule *genetic kod* or *DNA code* is called

To understand the nature of the genetic code, one must first know how many nitrogenous bases can define an amino acid. If each of the 20 amino acids were defined by one nitrogenous base, 20 nitrogenous bases would be needed. But there are only 4 nitrogenous bases in total. It follows that even a set (combination) consisting of two nitrogenous bases cannot determine 20 amino acids, because if nitrogenous bases form a set of 2, they can make a total of 16 sets ( $4^2 = 16$ ). If the nucleotides are combined in 3 pairs, 64 ( $4^3 = 64$ ) will form different sets and will be able to determine the arrangement of amino acids needed for the synthesis of the desired protein. A set of 3 such nitrogenous bases is called a triplet. Triplet means labeling amino acids with 3 nitrogenous bases. For example, AUU is leucine, GSS is valine, SAG is leucine. A part of the DNA chain consisting of 3 nitrogenous bases, which determines the sequence of amino acids in a protein molecule *a code* is called

After the essence of the genetic code was determined, it was necessary to find which triplet actually determined which amino acid. Such an important issue

was solved by American biochemists M. Nirenberg and J. Martey. In 1961, these scientists discovered the triplet that defines the amino acid phenylalanine, which consists of 3 uracils (UUU). Thus, the first triplet that determines phenylalanine - UUU was discovered. Later, triplets of other amino acids were also discovered.

In 1962, triplets of all 20 amino acids were found in the laboratories of M.Nerenberg and S.Ochoa. After all the triplets were found, it became clear that a single amino acid could be defined by 2, 3, 4, and even more triplets without being defined by a single triplet. For example: methionine is defined by one triplet (AUG), lysine is defined by 2 (AAA and AAG), isoleucine by 3 (AUU, AUS, and AUA), and serine by 4 triplets (USU, USS, USA, and USG).

To identify one amino acid with several triplets *code mirroring* is called Triplets do not overlap each other, that is, one triplet does not belong to another triplet, and each independently identifies only its own amino acids. There is no barrier separating the triplets.

Therefore, triplets are read along one line in the DNA chain only in one direction: ABC, ABC, ABC ABC, ABC... If one nitrogenous base is dropped in the DNA chain or another is added, the set of triplet in the DNA chain and their sequential arrangement changes along the chain. 3 of the 64 triplets are nonsense triplets (UAA, UGA, and UAG). The genetic code is the same (universal) in all organisms, that is, one triplet AAA determines the amino acid lysine in bacteria, plants, animals, and humans.

***Forensic biology of DNA in inspections importance.***In 1985, the English scientist A. Jeffreys identified a highly polymorphic family of minisatellite markers in the human genome and for the first time put forward the idea that the study of polymorphism of hypervariable (multiallelic) loci in the DNA (deoxyribonucleic acid) molecule can be important in forensic biological investigations. In the same year, for the first time, the possibilities of DNA-examination of material evidence were shown.

Since 1986, genetic methods have been used to solve identification tasks in forensic investigations of biological objects. The method of DNA examination is

also known as "genome dactyloscopy", "genotyping", "DNA analysis" (in English scientific literature - "DNA profiling", "DNA fingerprinting", "DNA typing"). DNA is the genetic code. Determining the unique characteristics of the genetic constitution of each person is the essence of this method.

Areas of the DNA molecule that are determined at the same place in a certain pair of human chromosomes and are responsible for the formation of a character are called homologous loci. The presence of homologous loci in different allelic states is related to the different sequence of nucleotides in them, and in this case the phenotypic signs of individuals are different from each other. Different alleles of a trait are always located at the same locus. Hypervariable (multiallelic) loci are part of the DNA molecule and have a different structure in most people. The same allelic variant of hypervariable genes can be found in unrelated individuals. Only the co-occurrence of these alleles in the human genome is specific to each individual. Differences in the sequence of nucleotides of different alleles at one locus provide uniqueness in DNA polymorphism. Examination of individual allelic variants is called genotyping.

Currently, nuclear and mitochondrial DNA tests are widely used in criminalistics. These inspections are carried out in the forensic examination of various physical evidences, unknown persons and dismembered corpses, in the examinations conducted in cases of child abduction or replacement, disputed paternity and other cases, identification (belonging to a specific person), diagnostic (biological parents , determining kinship) and classification (determining gender and race).

All tissues and fluids of the human body are subject to DNA testing. DNA tests can also be performed on biological material contaminated with various microflora, mixed nature (for example, human and animal tissues) and very small amounts of biological material.

DNA tests are multi-stage and somewhat complex, and these tests consist of the following algorithms:

- obtaining biological material for examination from the scene of the incident,

- injured and suspected persons with the participation of a specialist;
- extraction and purification of DNA from biological material;
  - concentration of DNA preparations;
  - qualitative and quantitative examination of extracted DNA;
  - amplification of specific areas of DNA using the polymerase chain reaction (PCR-polymerase chain reaction);
  - denaturation - breaking bonds in a double-stranded DNA molecule;
  - fragmentary analysis;
  - determining the genotype in the form of a spectrogram and a digital code using a special computer program and entering it into the database;
  - comparative genotypic analysis.

DNA testing requires samples belonging to this person or his relatives to determine whether an object belongs to a specific person. In the absence of such samples, it is possible to determine only the gender and race of the biological object, as well as whether they belong to one or more people.

The human DNA testing laboratory at the Republican Forensic Expertise Center named after Khadicha Sulaimanova, operating in Tashkent, is the only one in our country and the most prestigious institution in Central Asia.

Previously, DNA examination was conducted based on the decision of the investigator, investigator, prosecutor or judge, court ruling. Now the citizens themselves can also apply.

It is reported that Three samples are submitted for DNA testing - father, mother and child. In these investigations, there are mainly requests to clarify the fact of paternity. Blood and saliva are used as objects of examination. And the result will be provided within 30 days.

The methods and equipment used in these inspection processes are provided by the world's leading companies. Accordingly, the conclusion of DNA tests also shows the highest correct result.

Currently, the legal basis of the DNA database has been adopted in 44 countries. In all the laws, a great deal of attention is paid to ensuring the citizen's

right to data privacy, health and freedom, and most importantly, data security.

It is also worth mentioning that the Center of Forensic Expertise of the Republic named after Khadicha Sulaimanova is the first in Central Asia to perform the forensic biological examination of human DNA. began to be held.

## Control questions

1. When and by whom were DNA tests founded?
2. What are DNA tests based on?
3. What can be the object of DNA tests?
4. What questions are answered in DNA tests?
5. What is the algorithm of DNA tests?
6. Victims and suspects from the scene of the incident  
What is the procedure for obtaining biological material for examination?
7. How to extract and purify DNA from biological material  
will be held?
8. What is DNA drug concentration?
9. What are the qualitative and quantitative tests of the extracted DNA  
is included?
10. What is comparative genotypic analysis?
11. In the DNA test, the object belongs to a specific person  
What is the order of determination?
12. To the person undergoing DNA testing or his relatives  
For what purpose are relevant samples required?
13. What is meant by "homologous loci"?
14. In what cases are nuclear and mitochondrial DNA tests done?  
will be held?
15. What is meant by testing for individual allelic variants?

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