

# Forensic Medical Capacities of Research of Saliva Stains on Physical Evidence after Washing

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**Abstract**—Recent advances in genetics have allowed increasing acutely the capacities of the formation of reliable evidence in conducting forensic examinations. Thus, traces of biological origin are important sources of information about a crime. Currently, around the world, sexual offenses have increased, and among them are those in which the criminals use various detergents to remove traces of their crime. A feature of modern synthetic detergents is the presence of biological additives - enzymes. Enzymes purposefully destroy stains of biological origin. To study the nature and extent of the impact of modern washing powders on saliva stains on the physical evidence, specially prepared test specimens of different types of tissues to which saliva was applied have been examined. Materials and Methods: Washing machines of famous manufacturers of household appliances have been used with different production characteristics and advertised brands of washing powder for test washing. Over 3,500 experimental samples were tested. After washing, the traces of saliva were identified using modern research methods of forensic medicine. Results: The influence was tested and the dependence of the use of different washing programs, types of washing machines and washing powders in the process of establishing saliva trace and identify of the stains on the physical evidence while washing was revealed. The results of experimental and practical expert studies have shown that in most cases it is not possible to draw the conclusions in the identification of saliva traces on physical evidence after washing. This is a consequence of the effect of biological additives and other additional factors on traces of saliva during washing. Conclusions: On the basis of the results of the study, the feasibility of saliva traces of the stains on physical evidence after washing is established. The use of modern molecular genetic methods makes it possible to partially solve the problems arising in the study of unlaundered evidence. Additional study of physical evidence after washing facilitates detection and investigation of sexual offenses against women and children.

**Keywords**—Saliva research, modern synthetic detergents, laundry detergents, forensic medicine.

## I. INTRODUCTION

ONE of the components of social life safety is the safety of the sexual inviolability of the person. To date, the number of crimes against sexual freedom of a person's identity is maintained at a high level. In addition, there is a trend of rapid growth of sexual offenses involving children and adolescents. Sexual offenses are the most cynical form of crime against the person. They have a marked distorting effect on the psyche of the victims, increase the risk of suicidal behavior and violate the marital relationship [1]. All crimes of a sexual nature are high latency and often have mixed

character. More than a quarter of all women, mostly adolescents and youth, have been a victim of rape or attempted rape. However, only between 4% and 20% of victims reported the crime to police. In the structure of sexual crimes, rape is the most common at 56.2%, while combined forms of sexual violence, such as rape in conjunction with the combination of violent acts of a sexual nature constitute is 23.7%, while isolated sexual acts of violent nature are determined in 15.3% of cases [2]. According to statistical data released by the Police department of Kazakhstan, the number of sexual abuse cases is 0.9% and the number of attempted rapes is 3.9%. Most of the victims of sexual assault (73.7%) are aged between 7 years and 18 years. Among them, the share of victims aged 7-11 years is 29.8%, 12-13 years is 21.1%, and 14-17 years is 22.8%. Violent penile-vaginal contacts make up 79.5% of assaults, oral-genital accounts for 25.3%, and anogenital for 17.8%. Also 7.5% of rapists use condoms [2]. According to the literature [1], 20% of rapists have insufficient erections, and many of them cannot even finish the intercourse ejaculation. The main objective sign of past sexual intercourse by the mouth (Latin "per os"), is the detection of spots of saliva mixed with the semen or blood on clothes. In this connection, crucial in oral-genital contact, is the discovery of saliva. However, the fact of sexual violence can be confirmed only by the body of evidence [2]. Often these important sources of evidence, as the testimony of the victims and the accused in the course of the investigation lose their probative value because of their refusal to testify for various reasons or changing the initial testimony. In addition, sexual offenses, as a rule, take place without witnesses. Victims of sexual violence, especially children, are not always able to understand their feelings, and therefore, they can be sincerely errant about the nature of actions committed to them. Some of the victims may not understand the crux of the issue and what is at stake, and so may give the wrong answer [1], [3]. In this regard, the full realization of justice cannot be without identifying objective evidence of violence. The most important place in the investigation of sexual crimes is occupied by biological evidences of sexual abuse. These evidences carry information about the properties and characteristics of persons who have left it and about the nature of the committed acts. The discovery of traces of a biological nature is particularly important if there are no other or very little sources of information about the circumstances of the crime [3]. Ignorance of the capacities of a forensic medical examination of physical evidence may result irreversible loss of information available in the traces, especially in the investigation of non-obvious crimes, and also when there are

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not enough other sources of information about the circumstances of the crime [2], [3]. The totality of the evidence allows for taking the appropriate legal assessment in court. Contemporary manifestations of a crime are characterized by specific characteristics and changes in its structure. The most significant place in its structure is given to the activity aimed at the destruction of traces of the crime. A variety of scientific and technological achievements are successfully used in order to conceal the traces of the crime. Using simple measures such as the use of modern washing machines and washing powders may affect the results of a forensic medical examination. Synthetic detergents contain enzymes that destroy any biological body fluids [6]. Therefore, the need for research to establish the extent and the nature of their impact on the various stains of biological origin, including saliva on physical evidence has appeared [5]. Scientific and technological progress and advances in chemistry, physics and biology create opportunities for the use of new and effective methods of research evidence in forensic science. This makes it possible to obtain objective data for the findings of a forensic medical examination. Normally, saliva consists of water, organic substances and inorganic substances; it is readily soluble. Antigen-contained glycoproteins used in the analysis of saliva in forensic medicine are synthesized in the minor salivary glands and exactly match the blood group. Their content in saliva is 10-130 mg/dl [7]. To identify the saliva on physical evidence in forensic medical practice, serological, genotypic and immunological methods of investigation that allow working with the micro traces of biological origin are used [8]. Recently, the possibilities of studies using biological methods in forensic medicine were limited by definition of group and serum markers of erythrocyte systems [9]. Currently, research methods of deoxyribonucleic acid (DNA) - a molecular genetics examination, are increasingly used for the examination of any traces of biological origin [10]. DNA is the carrier of genetic information about an individual and serves as a source of genetically determined traits studied during the examination of biological objects [9], [10]. Features of traces research of biological origin for the purpose of identification and parentage by molecular typing methods are much more specific than traditional [11]. The versatility and high individual results make this method the most promising among all other methods of human identification in cases of the direct study of objects of biological origin [12]. Items containing genetic material (DNA) in the investigation of sexual crimes are often traces of saliva, sometimes mixed with blood or sperm, presented in the form of stains on the physical evidence [13]. However, the use of methods based on detection of specific DNA sequences of humans in forensic medicine has a number of features, and its use is associated with a number of challenges [14]. Thus, traces of biological origin, which are outside the body are subject to change, and DNA experiences degradation as a result of putrefaction or the devastating effects of enzymes, environmental factors, or contamination of foreign DNA [3]. Sometimes physical evidence with traces of biological origin, including saliva,

seized during a criminal investigation, is subject to washing with the use of modern washing powders. A characteristic feature of modern washing powders is the presence in their structure of biological additives, known as enzymes. The enzymes cleave and gradually destroy stains of different origin. Currently, almost all well-known brands of washing powders contain enzymes [4]. The amount of enzymes in each type of washing powder is different and depends on the brand of washing powder and manufacturer. Generally, enzymes used for production of washing powders include species of *Bacillus* bacteria. Waste products of these bacteria: proteases, amylases, lipases are included in washing powders. Each of these enzymes acts differently. In general, enzymes remove stains faster than conventional chemical cleaners [4]. Also, its effect is well expressed at low temperatures. Enzymes speed up chemical reactions, and ultimately, it has a significant effect on the results of further forensic examination of traces of biological origin. Significant prospects in the study of this issue emerged primarily through the use of the achievements of molecular genetics.

## II. AIMS AND OBJECTIVES

The aim of this paper is the study of the influence of the modern enzyme-contained washing powders on saliva stains on the physical evidence, as well as to research the effectiveness of using various forensic techniques and identification of opportunities of molecular-genetic examination in the study of physical evidence after washing.

## III. MATERIALS AND METHODS

Special experimental samples were prepared for the study of the nature of the influence of modern washing powders on saliva stains. For the preparation of samples the following fabrics were used: cotton, woolen, half-woolen, synthetic and semi-synthetic, and specifically the saliva of people with different blood groups of the AB0 system was applied to them. The saliva stains were dried. Then experimental samples with saliva stains were washed. The branded washing machines include Samsung, LG, Whirlpool, Electrolux, INDESIT, and the washing powders of well-known brands used include Ariel, Tide, Wisk Persil and Ecover Zero. After the test washing, experimental samples were examined using forensic biological and molecular genetic methods. A total of 3923 experimental sample and 52 objects of archival material were investigated by the continuous sampling method. Materials of forensic biological examinations of Karaganda were used as an archival material. The basis for its examination was the data received from the investigator, which indicated the fact of a prewash of the physical evidence. All the data were subjected to a comprehensive statistical processing using such applications as the spreadsheet of Microsoft Excel version 7.0 and Statistica 6.0 for Windows. The transition to the ranks was done in the study of some quantitative indicators. When describing data, the absolute and relative frequency (percentage share) were calculated.

Muller reaction on amylase in the L.O. Barsegyants's modification was used as an evidence method, testing for the presence of saliva. The main reagent in this reaction is Lugol solution. It was used for scoring the extracts from stains in a mixture with potato starch. In the presence of amylase in the examined samples, starch splitting occurs and the solution becomes transparent, it does not change its color. This reaction is characterized by high sensitivity. It allows for the examination of small sections of material with traces of various limitations. This reaction is absolutely specific and does not give a positive result in semen, blood and vaginal content. However, this reaction has disadvantages. It is extremely time-consuming, its implementation requires a certain amount of time, and the result must be confirmed with the use of diagnostic tests if you receive a negative result. Capacities of diagnostic tests are more significant, because they can be used directly at the scene of the crime. Immunoassay is reliable, specific, and its objectivity in the study of small or subjected to the destruction of saliva traces is very high. Application of this method allows to reveal traces of saliva in stains that were for a long time under the influence of adverse external factors, and to reduce the time of the study of an object for further investigation, which is especially important in cases of a small amount of the material [6]. Immunoassay of human saliva is based on the determination of  $\alpha$ -amylase in the test sample. The enzymes of amylase groups are the main subjects in the process of digestion. Thus,  $\alpha$ -amylase is an enzyme of salivary glands and pancreas. Two test systems are used during forensic biological examinations in Kazakhstan. One of them is the SERATEC Amylase Test, which is chromatographic immunoassay for the rapid detection of traces of human saliva. As active components, it contains two test monoclonal mouse anti-alpha amylase antibodies. The SERATEC Amylase Test confirms the presence of alpha-amylase of a person; it has a high sensitivity and specificity. The advantages of this test are its ease of use, reliability of results, as well as the high diagnostic sensitivity, lack of High-Dose-Hook effect, specificity and no influence on the result of examination of the blood, urine and seminal fluid of the person presented in the samples. Another test system is Rapid Stain Identification (RSID) Saliva kit for the detection of traces of human saliva by immunoassay. The format of immunochromatographic test - cassette allows to use a set of RSID Saliva as a fast, simple and reliable method for conducting forensic investigations of biological objects. The test has a high sensitivity and specificity, as it is based on the use of high-quality monoclonal antibodies against  $\alpha$ -amylase of the person. Then results of the RSID Saliva test correlate well with the intensity profile of STR markers in the further molecular genetic studies. The main features of the use of this kit are: a standard test format, easy interpretation of the results, the minimum time for the result, high specificity of the study, the lack of cross-reactivity with samples of biological fluids and saliva of animals, no hook effect at high concentrations of saliva. The test avoids false negative results, eliminates the need for re-examination and has high sensitivity (sustained response in the analysis of <50 nL of human

saliva). Sample processing provides the opportunity to further extraction of DNA for molecular genetic studies, and easy integration of the test in the scheme of laboratory analysis. In addition, immunochromatographic methods ensure high visibility of the results of the research; it can be used to design the necessary illustrations accompanying the conclusion of the expert. Isohemagglutinating serums such as anti-A, anti-B, anti-Colyclons A, anti-B and anti-H of different batches are used for studies of group affiliation of saliva by the AB0 system. For DNA purification standard protocols of different techniques (reagents Chelex 100 manufactured by Bio-Rad, using the DNA IQ™ System reagent kit manufactured by Promega (USA) and using a QIAamp DNA Investigator Kit columns manufactured by Qiagen) were used. To monitor possible contamination during DNA purification, a negative control was used. Evaluation of the quality and quantity of human DNA extracted from biological material was performed by using a set of reagents Quantifiler® Human DNA Quantification Kit in the system of determining the sequence ABI Prism™ 7500 Sequence Detection System manufactured by Applied Biosystems (USA). The study of DNA polymorphism was performed by polymerase chain reaction using a set of reagents AmpFISTR® Identifiler™ PCR Amplification Kit manufactured by Applied Biosystems (USA). The amplification conditions correspond to the recommendations of the amplification set. For evaluating of the specificity of the amplification reaction, the control DNA sample with known genotypic profile and a negative control without DNA was used. Separation and detection of fluorescently labeled amplified fragments was performed by capillary electrophoresis in an automatic analyzer, ABI Prism™ 3130 Genetic Analyzer. Analysis and accounting of the results of capillary electrophoresis was performed by using a software GeneMapper® ID Software v.3.2.

#### IV. RESULTS AND DISCUSSION

Studies have shown that in the cases of washing of physical evidence, the effect of washing powders on the saliva stains is signified. As a result, saliva stains on the physical evidence in the washing machine are subjected to mechanical effect that is rapid and more efficient than hand-washing. In addition, the stain is affected by the mechanical factor, warm or hot water and chemicals of washing powder. Surfactants in the washing powder in combination with enzymes entirely destroy biological stains. It was found that the use of any of washing powder recommended by the manufacturer specifically for the destruction of biological origin stains, according to the instructions and in accordance with the dose indicated on the packaging, in combination with the recommended washing program in an automatic washing machine leads to the almost complete destruction of the stains of saliva on clothing. In a subsequent study of these traces with the help of a forensic-quality biological reaction, the detection of traces of saliva is impossible for 100% of cases. Studies have shown that fresh saliva stains are easily soluble and completely removed even by washing in cold water. Using the immunoassay provides a weakly positive result in 5% of the study tracks. It is noted

that the possibility of obtaining a positive result is higher if the traces of saliva are present on natural fiber fabrics. The detection of saliva traces disposed on synthetic or semi synthetic tissues is impossible in 100% of cases. This depends on the fibers forming the fabric structure. Natural fibers have an uneven surface of fibers so that there is a mechanical delay of antigenic-contained saliva proteins on them. Synthetic fabrics have an absolutely smooth surface of fiber, so the ability to delay the saliva traces is completely absent. In this connection, the ability to detect residual amounts of saliva traces on natural fabrics is higher than synthetic fabrics. In the detected traces of saliva, in 1% of cases the group membership of saliva was identified and its genetic profile was set. In other cases, the amount of sufficient material to establish a group or DNA identification has not been received. This is due to the amount of DNA, which was below the sensitivity of the polymerase chain reaction. In this connection, the negative results were obtained. The combined effect of enzymes and other factors of washing lead to the DNA degradation. The structure of the DNA double helix bonded via hydrogen bonds is easily degraded by enzymes and heat. The research results of the expert material are the same as the experimental study. The research results obtained by experiment and the study of cases of physical evidence examination from the forensic practice can be estimated ambiguously. The results of the subsequent forensic examination depend on the type of woven garments, the brand of washing powder, and the type of automatic washing machine, and even the type of automatic washing program. Despite this, research has shown that the study of traces of saliva in stains on the physical evidence is appropriate even after washing. There is always the possibility of detecting residual amounts of saliva and its subsequent identification. Using molecular genetic methods gives additional possibilities in the study of small amounts of saliva. Analyzing the results of the study, it can be concluded on the mandatory study of physical evidence after washing.

#### V. CONCLUSIONS

1. In establishing the facts of destruction of traces of biological origin using the washing of physical evidence, the appointment of a forensic medical examination is necessary.
2. In the production of expertise, the expert is required to use the entire range of available modern research methods to resolve the raised issue; to use all the advanced capabilities of forensic laboratories for the completeness and comprehensiveness of the study during forensic medical examination.
3. The use of rapid tests in forensic biological expertise provides the forensic laboratory with rapid, accurate, sensitive, and inexpensive screening tools to determine the presence of human saliva in a sample.
4. Molecular genetic studies at the present stage of development are the only possible source of proving the traces being destroyed.
5. Enzyme-contained washing powders significantly affect objects of biological origin and affect the results of

forensic examinations. However, the use of innovative technologies in forensic practice allows for a positive result in the production of forensic examinations in cases of investigation of physical evidence after washing.

Thus, the low percentage of saliva detection in forensic biological research is due to objective reasons. The conditions of storage of physical evidence, the amount of time that has elapsed after the rape, and the use of external influencing factors, such as the washing of physical evidence, adversely affect the results of forensic examinations. However, using highly technical equipment can obtain a positive informative result. It should be noted that the removal of clothing of the victims and the alleged rapists after washing increases the chances of finding biological evidence of sexual abuse. This makes it possible to enhance the evidential base. The integrated approach to the selection and investigation of physical evidence in the investigation of sexual crimes can significantly increase the effectiveness of forensic medical examination in identifying biological traces of sexual violence and improve the quality of investigations. Getting even weak positive results collectively with other operational information obtained by investigative activities will help to confirm or to exclude the fact of sexual violation integrity of persons.

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