IDENTIFICATION OF SEMEN STAINS ON PHYSICAL EVIDENCE USING COMPREHENSIVE LABORATORY METHODS UNDER SIMULATED CONDITIONS

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Abstract: *This thesis highlights the diagnostic possibilities of complex laboratory methods used to identify semen spots on various object-carriers under conditions of modeled expertise. The importance of step-by-step examination of sperm traces based on visual, microscopic, biochemical, and immunological methods was analyzed. It has been established that the nature of the object-carrier, the shelf life of the stain, and environmental factors influence the laboratory test results. It has been shown that immunological studies based on the detection of prostate-specific antigen are of significant confirmatory importance in cases where spermatozoa are not detected morphologically.*

Keywords: *semen stain, physical evidence, carrier object, forensic biological examination, microscopic examination, acid phosphatase, prostate-specific antigen, PSA, enzyme-linked immunosorbent assay.*

ИДЕНТИФИКАЦИЯ ПЯТЕН СПЕРМЫ НА ВЕЩЕСТВЕННЫХ ДОКАЗАТЕЛЬСТВАХ С ИСПОЛЬЗОВАНИЕМ КОМПЛЕКСНЫХ ЛАБОРАТОРНЫХ МЕТОДОВ В ИМИТИРУЕМЫХ УСЛОВИЯХ

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Аннотация: *В данной диссертации освещены диагностические возможности комплексных лабораторных методов, используемых для выявления пятен спермы на различных объектах-носителях в условиях моделируемой экспертизы. Проанализирована значимость поэтапного исследования следов спермы с применением визуального, микроскопического, биохимического и иммунологического методов. Установлено, что на результаты лабораторных исследований влияют природа объекта-носителя, давность образования пятна и факторы окружающей среды. Показано, что иммунологические исследования, основанные на выявлении простатспецифического антигена, имеют важное подтверждающее значение в случаях, когда сперматозоиды не обнаруживаются морфологически.*

Ключевые слова: *пятно спермы, вещественное доказательство, объект-носитель, судебно-биологическая экспертиза, микроскопическое исследование, кислая фосфатаза, простатспецифический антиген, ПСА, иммуноферментный анализ.*

MODELLASHTIRILGAN SHAROITLARDA KENG QAMROVLI LABORATORIYA USULLARI YORDAMIDA ASHYOVIY DALILLARDAGI SPERMA DOG‘LARINI ANIQLASH

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Annotatsiya: *Ushbu dissertatsiya modellashtirilgan ekspertiza sharoitida turli xil obyekt-tashuvchilarda sperma dog‘larini aniqlash uchun qo‘llaniladigan kompleks laboratoriya usullarining diagnostik imkoniyatlarini yoritib beradi. Sperma izlarini vizual, mikroskopik, biokimyoviy va immunologik usullar asosida bosqichma-bosqich*

tekshirishning ahamiyati tahlil qilingan. Laboratoriya sinovlari natijalariga obyekt-tashuvchining tabiati, dog'ning saqlanish muddati va tashqi muhit omillari ta'sir ko'rsatishi aniqlangan. Prostataga xos antigenni aniqlashga asoslangan immunologik tadqiqotlar spermatozoidlar morfologik jihatdan topilmagan hollarda muhim tasdiqlovchi ahamiyatga ega ekanligi ko'rsatilgan.

Kalit so'zlar: *sperma dog'i, ashyoviy dalillar, obyekt-tashuvchi, sud-biologik ekspertizasi, mikroskopik tekshiruv, kislotali fosfataza, prostataga xos antigen, PSA, immunoferment tahlili.*

Relevance of the topic: Identifying biological traces left on clothing, bed linens, napkins, paper, and other objects during the investigation of crimes against sexual integrity is one of the important tasks of forensic medical examination. In such cases, discovering semen stains and confirming their biological nature allows for the reconstruction of the crime mechanism, the identification of suspects, and the extraction of evidential material for subsequent molecular genetic testing. The traditional microscopic method is based on the detection of spermatozoa. However, the effectiveness of this method may decrease in cases of azoospermia, oligospermia, vasectomy, stain aging, or the degradation of spermatozoa under the influence of the external environment. Therefore, the examination of semen stains using a combination of visual, morphological, biochemical, and immunological methods is of high relevance.

Purpose of the study: To evaluate the possibilities of identifying semen stains on various physical evidence under simulated conditions using comprehensive laboratory methods and to develop an optimal algorithm for forensic biological examination.

Introduction: One of the important tasks of forensic biological examination is the study and identification of the presence of sperm in the objects under investigation using precise and reliable methods. This is especially important in the investigation of cases of a sexual nature, where the evidence is often in the form of old, degraded or low-contrast traces. Under these conditions, the reliability of the result largely depends on the correct choice of analysis method and the method's resistance to the effects of time, storage conditions, and biomaterial degradation. The presented work provides a comprehensive assessment of the effectiveness of four laboratory methods for sperm identification used in forensic biological practice. The study included 704 sperm samples dried in gauze with various shelf lives (from 1 to 5 years). Enzyme-linked immunosorbent assay systems were used to determine the concentration of free and total prostate-specific antigens. At the same time, samples were examined in the laboratory using the A.K. Seropyan microscopy method, paper chromatography, and prostate-specific antigen rapid tests. The dried human sperm samples above were stored for various periods under laboratory conditions at room temperature, i.e., +18...+25 °C, with relative humidity of 40–60%, without direct sunlight and special climate control. ° These conditions allow for the reliable modeling of the real environment prior to the testing of physical evidence.

Each method involves quantitative and qualitative evaluation of the results, including the absolute number of positive reactions and their percentage relative to the total sample. Such statistical analysis allows for determining not only the sensitivity of the methods but also the stability of the sample's biological properties over time.

Detection of prostate-specific antigen in liquid sperm

PSA Indicator	Range (Min – Max)	Average Value
Total PSA	120 540 – 827 640 ng/ml	442 202 ng/ml
Free PSA	65 090 – 454 800 ng/ml	228 319 ng/ml

Control studies were conducted to determine the amount of prostate-specific antigen in human seminal fluids under normal conditions. The study included 10 previously known samples taken from voluntary donors. Before obtaining sperm samples, the donors underwent a medical examination for the absence of relevant urological diseases. The levels of total and free prostate-specific antigens were determined using the aforementioned set of ELISA methods.

Amounts of total and free prostate-specific antigens in the liquid semen taken as a sample

№	Sample	Liquid state total PSA	Liquid state Free PSA	Total PSA after drying	Free PSA after drying
1	№1	534500	290600	29120	31470
2	№2	667200	320000	21550	28640
3	№3	827640	454800	46460	57525
4	№4	474620	251500	24980	23950
5	№5	120540	65090	7530	7650
6	№6	348220	167100	17410	18560
7	№7	236850	118400	12460	12080
8	№8	568700	295700	33450	33980
9	№9	245230	112800	13400	13920
10	№10	398520	207200	33210	22520

A general analysis of the table data shows that in the 10 samples included in the study, the indicators of the total and free prostate-specific antigen (PSA) in the liquid state and after drying differed significantly. In the liquid state, the total PSA content ranged from 120,540 to 827,640 and the free PSA content from 65,090 to 454,800. The highest values of total and free PSA were found in sample No3, and the lowest values in sample No5. This indicates that the PSA content in the initial biological samples varies depending on individual characteristics, sperm composition, and sample concentration. The average amount of total PSA in the liquid state across all samples was 442,202, while the average amount of free PSA was 228,319 units. Consequently, before drying, the total PSA content in the samples was higher than that of the free PSA. Free PSA in the liquid state averaged 51.6% of total PSA. Despite the quantitative differences between the samples, a general pattern is observed where both types of PSA are detected at a high level in the initial liquid material. A sharp decrease in total and free PSA values was noted after the samples were dried. In the dried samples, the total PSA content ranged from 7,530 to 46,460, while the free PSA content ranged from 7,650 to 57,525. Even after drying, the highest total and free PSA values were maintained in sample No3, while the lowest values were maintained in sample No5. This indicates that a relatively large portion of the antigen is detected even after drying in samples with initially high PSA levels. In the dried samples, the average amount of total PSA was 23,957, and the average amount of free PSA was 25,029.5. Compared to the results in the liquid state, the total PSA indicator during the drying process decreased by an average of 94.6%, while the free PSA indicator decreased by 89.0%. In individual samples, the reduction rate of total PSA ranged from 91.7 to 96.8%, and the reduction rate of free PSA from 87.4 to 91.0%. Thus, the drying process had a significant effect on the content of both biochemical indicators, but the relative decrease in free PSA was slightly lower than in total PSA.An important aspect of the obtained results is that although the PSA content decreased sharply after drying, it was determined by the laboratory method in all 10 samples. This indicates that the prostate-specific antigen persists to a certain extent even in dried semen stains. Therefore, immunological and enzyme-linked immunosorbent assays based on PSA detection can be of significant diagnostic importance for identifying dried semen spots on physical evidence. Especially in cases where spermatozoa are not detected during morphological examination, are fragmented, or have lost their structure under the influence of the external environment, determining PSA serves as an additional laboratory criterion confirming the presence of sperm plasma. Thus, the overall results in the table show that drying significantly reduces the amount of total and free PSA in semen, but does not lead to the complete loss of antigens. The relatively better preservation of free PSA after drying indicates that it can be used as a promising additional marker in the study of dried and stored semen stains. However, the PSA test serves to determine the biological material's belonging to the sperm plasma and does not determine the trace's belonging to a specific individual. Therefore, in forensic biological examination, PSA results should be evaluated comprehensively with the results of microscopic, biochemical, and molecular genetic studies.

Amount of total and free prostate-specific antigen in the semen sample after drying

т/р	Indicator	M	м	Me	Min-Max	VK, %
1	Liquid state total PSA	442202,0	68300,8	436570,0	120540–827640	48,85
2	Liquid state free PSA	228319,0	37252,0	187150,0	65090–454800	51,60
3	Total PSA after drying	23957,0	3759,1	23265,0	7530–46460	49,62
4	Free PSA after drying	25029,5	4512,7	21235,0	7650–57525	57,02

The table presents the arithmetic mean (M), error of the mean (m), median (Me), minimum and maximum values, as well as the coefficient of variation (VK) of the total and free prostate-specific antigen (PSA) indicators in the liquid state and after drying for 10 samples. The results show that PSA levels varied across a wide range in all examined samples. The mean amount of total PSA in the liquid state was 442,202.0±68,300.8, and the median was 436,570.0. The lowest value of this indicator was 120,540; the highest was 827,640; and the coefficient of variation was 48.85 percent. The proximity of the mean to the median indicator indicates that the central distribution of the results is relatively proportional. However, the large difference between the minimum and maximum values and the high coefficient of variation mean that there is significant individual variability in the total PSA content of the samples. The mean free PSA content in the liquid state was 228,319,0± 37,252.0, with a median of 187,150.0. Its minimum value was recorded at 65,090 and maximum value at 454,800. The coefficient of variation was 51.60%, indicating a high degree of dispersion even in free PSA indicators in the liquid state. The fact that the mean is higher than the median means that higher scores in some samples influenced the overall mean. After drying the samples, the average amount of total PSA decreased to 23,957.0±3,759.1. The median was 23,265.0, and the minimum and maximum values were 7,530 and 46,460, respectively. The coefficient of variation was 49.62%. The proximity of the mean and median indicates that the central distribution of total PSA results in the dried samples is relatively uniform. Nevertheless, the high coefficient of variation indicates that the degree of PSA preservation varied between the samples. After drying, the mean free PSA content was 25,029.5±4,512.7, and the median was 21,235.0. The indicators ranged from 7,650 to 57,525. The coefficient of variation was 57.02%, which was the highest among the four indicators in the table. This indicates that the degree of free PSA retention after drying is highly variable across samples. The fact that the mean value is higher than the median can be explained by the relatively high content of free PSA in some samples. A comparison of the results in the liquid and dried state shows a sharp decrease in PSA levels during the drying process. In particular, the average value of the total PSA decreased from 442,202.0 to 23,957.0, i.e., by approximately 94.6%. The mean value of free PSA decreased from 228,320.0 to 25,029.5, i.e., by approximately 89.0%. Thus, drying had a stronger effect on the amount of total PSA compared to free PSA. The relatively low degree of reduction of free PSA indicates that it may be more resistant to the effects of drying to a certain extent. The coefficient of variation in all groups ranged from 48.85 to 57.02 percent. The fact that these indicators exceed 33 percent indicates a high degree of variability in the research results. Such dispersion may be due to differences in the initial concentration of sperm samples, individual biological characteristics, drying conditions, shelf life, extraction efficiency, and the laboratory determination process. Overall, although the quantitative indicators of total and free PSA decreased sharply as a result of drying, both markers remained at a detectable level in the dried samples. This indicates that enzyme-linked immunosorbent assay (ELISA) based on PSA detection can be used in the forensic biological examination of dried semen stains. Complex determination of total and free PSA can be of additional diagnostic importance, especially in cases where sperm are not detected during morphological examination or are destroyed as a result of external influences. However, the table presents only descriptive statistical indicators. Therefore, to evaluate the difference between the results before and after drying as statistically significant, it is necessary to calculate the p-value using a statistical criterion corresponding to paired samples.

Conclusion: The results of the study conducted under simulated examination conditions demonstrated the effectiveness of a comprehensive laboratory approach in identifying semen stains on physical evidence. The step-by-step examination of semen traces using visual, microscopic, biochemical, chromatographic, and immunological methods increases the reliability of the obtained results. Although the levels of total and free prostate-specific antigen decreased sharply during the drying process, PSA remained at detectable levels in all dried samples. Total PSA levels decreased by an average of 94.6%, and free PSA by 89.0%. These results indicate that PSA, especially free PSA, can serve as an important additional diagnostic marker in the examination of semen stains that have been dried and stored for a certain period. Enzyme-linked immunosorbent assay (ELISA) based on PSA detection allows for the confirmation of the presence of seminal plasma even in cases where spermatozoa are not detected microscopically, are fragmented, or have lost their morphological characteristics. However, a positive PSA result does not identify the specific individual to whom the biological trace belongs. Therefore, its results must be evaluated comprehensively alongside microscopic, biochemical, and molecular-genetic examinations. Overall, this approach serves to increase the sensitivity, objectivity, and evidentiary value of forensic biological examinations.

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