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Features of establishing the presence of blood in old stains by the method of thin-layer vertical chromatography

Due to the fact that the bloodstain pattern analysis takes the first place in the structure of the study of biological objects, the question of the study of blood patterns is relevant today. The main task (that will allow to solve the following, which puts the investigation before the experts) is to prove that these patterns, which are examined, contain blood, (regardless of their remoteness, attempts to destroy patterns of blood), define its types or refute the presence of blood in blood. If the expert fails to prove the presence of blood, he must refuse to address the following issues. Otherwise, the expert may obtain results that will ultimately lead him to an erroneous conclusion.

The article considers options for extraction of old blood stains in order to establish the presence of blood by the method of thin-layer vertical chromatography. This question is still relevant, as biological material can change under the influence of environmental factors and time and this can be an obstacle to establishing the fact of its presence on physical evidence, and also it can do harm the quality of the study and the correctness of conclusions, made as a result of the study. In order to select substances that could provide rapid extraction of old blood stains, a study was performed using weak solutions of alkalis and acids and subsequent determination of the presence of blood by thin layer vertical chromatography. In immunological studies, there have been cases where blood crusts on non-hygroscopic surfaces under the influence of natural factors (such as high temperatures and direct sun rays) have not been extracted in saline and distilled water for several days. In addition, when using known blood samples in the reactions, the dependence of the saturation of the extracts on the age of the samples and the time of their extraction. These observations prompted researches in the sector of biological research and accounting of the Cherkasy NDEKs of the Ministry of Internal Affairs on methods for extracting

old blood stains, which would allow to detect blood in the shortest possible time by the method of thin-layer vertical chromatography.

Keywords: *old blood stains, thin-layer vertical chromatography, forensic expertise, haemoglobin, extracting.*

Formulation of Research Problem. Every year the number of crimes against a person increases significantly. Accordingly, there is a need for rapid and high-quality investigation of physical evidence and objects based on the combined use of data from science, criminalistics and other areas of expert research.

Traces of biological origin are strong material evidence not only in criminal proceedings for murder, grievous bodily harm, robbery, rape, robbery, but also in other crimes committed with human participation ¹. Due to the fact that the bloodstain pattern analysis takes the first place in the structure of the study of biological objects, the question of the study of blood patterns is relevant today ².

The main task (that will allow to solve the following, which puts the investigation before the experts), is to prove that these patterns, which are examined, contain blood, (regardless of their remoteness, attempts to destroy patterns of blood), find out its types or refute traces of blood. If the blood belongs to a person, then establish the possibility of its origin from a particular person, which is primarily determined by identifying the group affiliation by the system AB0 ³.

If the expert fails to prove the presence of blood, he must refuse to solve the following issues. Otherwise, the expert may obtain results that will eventually lead him to an erroneous conclusion ⁴.

Therefore, the expert must clearly, and most importantly — reasonably, using modern methods, to decide on the presence or absence of blood at the facilities provided for examination ⁵.

Any biological material seized at the scene or crime scene (including blood) may be altered or even destroyed in the event of improper removal or non-compliance with the packaging conditions. It is important to store seized biological traces, for which measures are taken to create artificial conditions that

¹ Фурман Я. В., Юсупов В. В., Котляренко Л. Т., Дмитрук Р. С. Особливості збирання у досудовому провадженні біологічних слідів людини : метод. рек. Київ, 2016. 44 р.

² Кривда Р. Г., Юшук К. М., Стоєва М. І. Дослідження можливості використання імунохроматографічного тесту «Cito Test Fecal Occult Blood» для встановлення наявності та видової приналежності слідів крові на речових доказах при проведенні комплексної судово-медичної експертизи. *Молодий вчений*. 2017. № 4. Р. 44.

³ Бурчинський В. Г., Дем'янчук А. П., Хохолєва Т. В. Дослідження рідкої крові та її слідів на речових доказах : метод. рек. Київ, 2010. 32 р.

⁴ Туманов А. К. Судебно-медицинское исследование вещественных доказательств. Москва, 1961. 580 р.

⁵ Дяченко Н. М., Єрмолаєва А. О., Чепіга С. М. Судово-медичні імунологічні дослідження слідів крові та виділень : зб. метод. рек. Київ, 2005. 70 р.

prevent the action of natural factors that can destroy the structure of biological objects ¹.

Analysis of Essential Researches and Publications. Determination of the presence of blood by thin-layer vertical chromatography is one of the most common and convenient methods. There is no information in the literature on the peculiarities of extraction and further study of old blood stains, which for various reasons are difficult to extract.

The **Article Purpose** is to identify substances that could provide rapid extraction of old blood stains for further use of extracts to determine the presence of blood by thin-layer vertical chromatography.

Main Content Presentation. During immunological studies there were cases when blood crusts on non-hygroscopic surfaces have not been extracted in saline and distilled water for several days under the influence of natural factors (such as high temperatures and direct sunlight).

In addition, when using known in the laboratory blood samples in the reactions revealed the dependence of the saturation of the extracts on the age of the samples and the time of their extraction. These observations prompted the staff of the sector of biological research and accounting at Cherkasy RECC of the MIA of Ukraine to investigate the methods for extracting old blood stains, which would allow to detect blood in the shortest possible time by the method of thin-layer vertical chromatography.

The thin-layer chromatography is an evidence-based method for detecting haemoglobin or its derivatives. This method has a number of advantages, as it helps to maximize the preservation of information about the detected blood for further study (determination of blood protein, detection of group antigens, DNA analysis, etc.) and the rational use of this material ².

Haemoglobin is a mandatory component of erythrocyte stroma. It belongs to the protein compounds, contains the protein globin and a colored prosthetic group — heme ³.

Determination of the presence of blood is based on the peroxidase properties of haemoglobin, which (similar to peroxidase enzymes) has the ability to catalyze redox reactions. Haemoglobin decomposes hydrogen peroxide with the release of atomic oxygen, which, in turn, oxidizes benzidine, accompanied by discoloration of the developers. The positive result of the reaction is evidenced by the appearance of blue zones on the plate with $R_f = 0.82-0.9$.

¹ Фурман Я. В., Юсупов В. В., Котляренко Л. Т., Дмитрук Р. С. Особливості збирання у досудовому провадженні біологічних слідів людини : метод. рек. Київ, 2016. 44 р.

² Бурчинський В. Г., Дем'янчук А. П., Хохолєва Т. В. Дослідження рідкої крові та її слідів на речових доказах : метод. рек. Київ, 2010. 32 р.

³ Кривда Г. Ф., Дем'янчук А. П., Котельникова В. О., Старовойтова Р. О., Кривда Р. Г. Судово-медичне дослідження речових доказів : навч.-метод. посіб. Херсон, 2014. 460 р.

In the guidelines, it is recommended to extract the objects with distilled water or saline solution of sodium chloride at a temperature of +4 to +8 °C. The extraction time depends on the solubility of the stain substance and can last from 1 hour to 2-3 days (the optimal extraction time is 18-20 hours).

If bloodstains on physical evidence have a significant history of formation (3-6 months or more) or were subject to the fixing action of sunlight, chemicals, alcohols, etc., the extraction can take place at room temperature, but not higher than 22 °C¹.

During the study of old blood samples, it was found that with the help of the above-mentioned recommendations to achieve saturation of the extract and obtain on the chromatographic plate spherical blue stains with $R_f = 0.82-0.9$, which corresponds to R_f blood, failed. Given the protein nature of haemoglobin, the possibility of influencing its structure with solutions of weak acids (acetic acid) and weak alkalis (10% ammonia solution) was considered.

Materials and methods of research. The research was conducted in 2020-2021 on the basis of the laboratory of the sector of biological research and accounting at Cherkasy RECC of the MIA of Ukraine. To achieve this goal, the blood samples, taken in 2011 from living people, without the addition of anticoagulants, clot activators, etc., poured on sterile gauze wipes and dried in sterile laboratory conditions with temperature and humidity, which meets the requirements of the biological laboratory, without direct sunlight, were used. Subsequently, blood samples dried on sterile gauze wipes were placed in paper rolls packed in paper envelopes. The prepared samples were stored in cabinets in the laboratory.

The method of detecting traces of blood contains the following stages:

- preparation of clippings from old blood samples;
- preparation of clippings from controls of objects-carriers and gauze;
- preparation of clippings from fresh blood samples;
- preparation of 0.01% solution of erythrocytes - *witness*;
- preparation of 10% ammonia solution;
- preparation of 10% acetic acid solution;
- extraction of clippings;
- research by thin-layer vertical chromatography.

Results of the research

Extraction method

For extraction, four clippings were prepared from obsolete traces of blood, from fresh blood samples and controls of carriers to them, which were placed in test tubes and separately filled (with a slight excess) with distilled water, saline, 10% ammonia solution and 10% acetic acid solution. The tubes were closed and extracted for 18 hours in a household refrigerator at a temperature of +6 °C.

¹ Кривда Г. Ф., Дем'янчук А. П., Котельникова В. О., Старовойтова Р. О., Кривда Р. Г. Ор. cit.

Research by thin-layer vertical chromatography method

The obtained extracts were centrifuged for 5 min at 1500 rpm and examined by thin-layer vertical chromatography method. The distribution was performed on a plate *Sorbfil PTSH-P-A-UV* length of 100 mm in a system of solvents n-butanol — glacial acetic acid — distilled water (4: 1: 2) using as a *witness* 0.01 % solution of pre-known blood.

One drop of each extract was applied in layers to the plate. Additionally, 10% ammonia solution, 10% acetic acid solution and physiological saline were applied to the plate for control. The plates were then heated for 15 min at 100 °C and placed in a solvent chamber. The plate was removed when the solvent level did not reach 10 mm to its upper edge, dried at room temperature to eliminate the smell of acetic acid, and then treated successively with 1% alcohol solution of benzidine and 3% hydrogen peroxide solution and determined *Rf witness* and extracts.

As a result of the action of developers, blue stains of spherical shape with $Rf = 0.89$, which corresponded to *Rf witness*, observed:

- with extracts from a fresh blood sample extracted in distilled water, saline, 10% ammonia solution and 10% acetic acid solution;
- with extracts from old blood samples extracted in 10 % ammonia solution and 10% acetic acid solution.

Controls of object-carriers extracted in distilled water, saline, 10% ammonia solution and 10% acetic acid solution gave a negative reaction result. Also, a negative reaction result was obtained with old blood samples, which were extracted in distilled water and in saline.

The extraction of the test samples was extended for another 24 hours, after which the extracts were centrifuged again and the research was performed by thin-layer vertical chromatography method under the above conditions. The obtained results corresponded to the previous ones, the indicators of extracts of old blood samples in distilled water and in physiological solution did not change, i.e., no blue spherical stains with *Rf* corresponding to *Rf witness* were observed on the chromatographic plate.

The extraction was extended to 20 days, until the old blood samples appeared on the chromatographic plate as blue spherical stains with *Rf*, which corresponds to *Rf witness*. The stains were not expressed: the detection of stains is almost on the verge of detection.

The results of the study are given in table. 1.

Table 1

SN (№)	Studied extract	Day									
		1st	2nd	3rd	6th	9th	10th	14th	16th	18th	20th
1	Witness 0,01 %-and Rf = 0,89	+	+	+	+	+	+	+	+	+	+
2	Old blood sample with saline	-	-	-	-	-	-	-	-	-	-/+
3	Old blood sample with distilled water	-	-	-	-	-	-	-	-	-	-/+
4	Old blood sample with 10% ammonia solution	+	+	+	+	+	+	+	+	+	+
5	Old blood sample with 10% acetic acid solution	+	+	+	+	+	+	+	+	+	+
6	Fresh blood sample with saline	+	+	+	+	+	+	+	+	+	+
7	Fresh blood sample with distilled water	+	+	+	+	+	+	+	+	+	+
8	Fresh blood sample with 10% ammonia solution	+	+	+	+	+	+	+	+	+	+
9	Fresh blood sample with 10% acetic acid solution	+	+	+	+	+	+	+	+	+	+
10	Control of the object- carrier with saline	-	-	-	-	-	-	-	-	-	-
11	Control of the object- carrier with distilled water	-	-	-	-	-	-	-	-	-	-
12	Control of the object- carrier with 10% ammonia solution	-	-	-	-	-	-	-	-	-	-
13	Control of the object- carrier with 10% acetic acid solution	-	-	-	-	-	-	-	-	-	-

SN (№)	Studied extract	Day									
		1st	2nd	3rd	6th	9th	10th	14th	16th	18th	20th
14	Control of 10% ammonia solution	—	—	—	—	—	—	—	—	—	—
15	Control of 10% acetic acid solution	—	—	—	—	—	—	—	—	—	—
16	Saline control	—	—	—	—	—	—	—	—	—	—
17	Gauze control	—	—	—	—	—	—	—	—	—	—

Explanation of Symbols:

«—» — stain is not detected;

«+» — stain is detected;

«-/+» — stain is detected on the verge of detection.

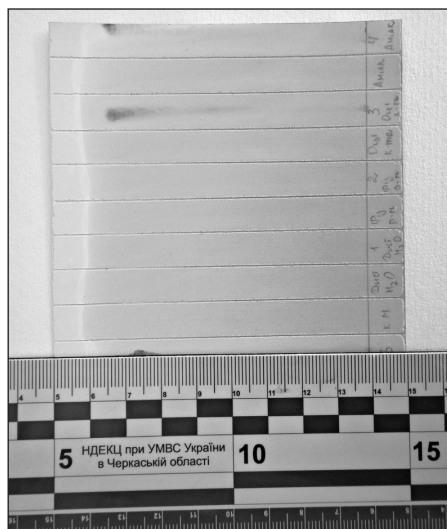


Fig. 1. Figure of the chromatographic plate with the studied objects after the first day of extraction

As a result of the study, it was found that in the case of extraction of:

- old blood samples (dried 9 years ago) with distilled water and saline to form blue spherical stains on a chromatographic plate with $R_f = 0.89$, which corresponds to R_f witness, only on the 20th day of extraction;

- old blood samples (dried 9 years ago) with 10% ammonia solution and 10% acetic acid solution formation of spherical blue stains on the chromatographic plate with $R_f = 0.89$, which corresponds to R_f witness, obtained after the first day of extraction;
- fresh blood samples with distilled water and saline to form blue spherical stains on a chromatographic plate with $R_f = 0.89$, which corresponds to R_f witness, obtained after the first day of extraction;
- controls of object-carriers with distilled water, saline, 10% ammonia solution and 10% acetic acid solution obtained a negative reaction result, i.e. blue stains of spherical shape were not formed.

Conclusions

It was found that the determination of the presence of blood by thin-layer vertical chromatography after conventional extraction of objects with saline or distilled water in old blood samples is complicated by long-term extraction.

If weak solutions of alkalis and acids (such as 10% ammonia solution and 10% acetic acid solution) are used, positive results are obtained after the first day of extraction.

The obtained extracts with 10% ammonia solution and 10% acetic acid solution require further research – to establish their suitability during other immunological tests, in addition to determining the presence of blood.

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Особливості визначення наявності крові

в застарілих плямах методом

тонкошарової вертикальної хроматографії

Розглянуто варіанти екстрагування застарілих плям крові з метою визначити наявність крові методом тонкошарової вертикальної

хроматографії. Це питання є актуальним, оскільки біологічний матеріал має здатність змінюватися під впливом чинників зовнішнього середовища й часу, що може стати на заваді виявленню його наявності на речовому доказі, а також якості проведеного дослідження та правильності зроблених у результаті дослідження висновків. Із метою добору речовин, які б могли забезпечити швидке екстрагування застарілих слідів крові, проведено дослідження з використанням слабких розчинів лугів і кислот та подальшим визначенням наявності крові методом тонкошарової вертикальної хроматографії.

Ключові слова: застарілі сліди крові, тонкошарова вертикальна хроматографія, судова експертиза, гемоглобін, екстрагування.

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Особенности установления наличия крови в застарелых пятнах методом тонкослойной вертикальной хроматографии

Поскольку экспертиза следов крови занимает первое место в структуре исследования биологических объектов, в настоящее время актуален вопрос исследования следов крови. Основная задача (позволяющая решить вопросы, которые следствие ставит перед экспертами) — это доказать, что в представленных на экспертизу следах имеется кровь (независимо от их давности и/или попытки их уничтожить) и выявить её видовую принадлежность или опровергнуть наличие крови в следах. Если эксперту не удастся доказать наличие крови, то он должен отказаться от решения следующих вопросов. В противном случае эксперт может получить результаты, которые в конечном итоге приведут его к ошибочному выводу.

В статье рассмотрены варианты экстрагирования застарелых пятен крови для установления наличия крови методом тонкослойной вертикальной хроматографии. Этот вопрос весьма актуален, поскольку биологический материал может изменяться под воздействием факторов внешней среды и времени, что может помешать установить факт его наличия на вещественном доказательстве, а также навредить качеству проведённого исследования и правильности сделанных в результате исследования выводов. Для подбора веществ, которые могли бы обеспечить быстрое экстрагирование застарелых следов крови, провели исследование с использованием слабых растворов щелочей и кислот и последующим установлением наличия крови методом тонкослойной вертикальной хроматографии. В ходе иммунологических исследований случалось, что корочки крови на негигроскопичных поверхностях под воздействием природных факторов (таких, как высокие температуры и прямые солнечные лучи) не подвергались экстрагированию в физиологическом растворе и дистиллированной воде в течение нескольких суток. Кроме того, при использовании известных образцов крови в реакциях выявили зависимость насыщенности экстрактов от давности образцов и от времени их

экстрагирования. Эти наблюдения сподвигли сотрудников сектора биологических исследований и учёта Черкасского НИЭКЦ МВД Украины исследовать способы экстрагирования застарелых пятен крови, которые позволяли бы выявить кровь в кратчайшие сроки методом тонкослойной вертикальной хроматографии.

Ключевые слова: застарелые следы крови, тонкослойная вертикальная хроматография, судебная экспертиза, гемоглобин, экстрагирование.

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Contributors

The authors contributed solely to the intellectual discussion underlying this paper, case-law exploration, writing and editing, and accept responsibility for the content and interpretation.