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Evaluation of the usefulness of the alternative light source (ALS) in differentiating simulated bloodstains

Ocena przydatności alternatywnego źródła światła (ALS) w różnicowaniu pozorowanych śladów krwi

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Introduction: The alternative light source (ALS) is a helpful technique for the detection of biological traces at a crime scene, which allows preservation of the material without destroying it. The aim of this study was to differentiate the human blood from a group of simulated bloodstains, which included: red borscht, raspberry juice, cherry liqueur, cranberry juice, tomato bruschetta, tomato paste, raspberry jam, rust, red spray, red wine and tomato ketchup.

Material/Methods: Stains, made of different types of material, were illuminated with the ALS emitted by the Mini-CrimeScope 400 (SPEX Forensics) with yellow, dark yellow, orange and red filters. The results of the analysis were presented as a description and also documented in photographs.

Results: The usage of light sources without color cut-off filters does not allow us to differentiate unequivocally real bloodstains from the trace evidence imitating or resembling bloodstains. The usage of different color cut-off filters (especially red filter) allowed us to exclude simulated bloodstains made of food and alcohol by using CSS light and light with a wavelength of 535 nm, 515 nm, 455 nm, 415 nm and 300-400 nm.

Discussion: Due to the different optical properties of blood and substances containing vegetable ingredients, forensic experts can differentiate human blood from simulated bloodstains by using the ALS in a non-destructive and quick way already at the crime scene. The ALS may be an example of a method which can replace more commonly used chemical-based screening tests.

Keywords: **bloodstains • alternative light source • simulated bloodstains • fluorescence • light absorption**

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INTRODUCTION

Bloodstains are one of the most common body fluids found at crime scenes. Blood traces may be detected visually without additional methods or by using various techniques. Most frequent methods that use additional techniques include:

- optical with UV, IR, visible light [6];
- chemical with luminol, test papers [19];
- imunochromatografic tests [14];
- combined, optical and chemical methods [23].

These methods can be also divided into specific or non-specific methods. Specific methods are based on the particular identification of a biological trace in the laboratory, while non-specific methods are based on an unclear identification of biological traces at the crime scene. Non-specific methods, like hydrogen peroxide testing [3], color tests, which use the presence of peroxidase [17], and luminol test [24], usually do not require the usage of additional methods to reveal the blood. The choice of method is determined by: the type of the tested trace, its size and quantity, subsoil on which trace was found, and also the elapsed time between the crime and moment of the investigation of the crime scene [8, 12, 18, 25].

Due to the fact that the bloodstains are the carrier of genetic information, the tests leading to the exposure and preservation of the traces are very important, and often the only proof that allows to associate the perpetrator with a crime. From a historical perspective, a large number of methods and protocols for blood detection were developed, each of them having advantages and disadvantages. It usually happens that at the crime scene there is just a small amount of biological evidence, so it is significant not to destroy it by technics used during criminal investigation. Mostly bloodstains are visible to the naked eye, and their features such as color and texture, enable an initial identification. The test should be non-destructive, so the DNA can be preserved [22].

One of the simplest tests used to detect bloodstains is an alternative light source (ALS) [10, 22]. The ALS is a non-invasive and non-destructive method based on the absorptive and photoluminescent qualities of the examined trace evidence [21]. Fluorescence is the property of absorbing light of a lower wavelength and emitting light of a greater wavelength [10]. An investigated area should be scanned with the ALS before the application of the other reagents [20].

Blood absorbs shorter wavelengths and reflects longer wavelengths in the range of 635–700 nm. Visualization of blood can be intensified by exposing the area to wavelengths in the blue or violet spectrum (400-480 nm). These wavelengths are absorbed by the blood and only a small fraction of light is reflected, so the illuminated area appears significantly darker [1]. In a fresh bloodstain, hemoglobin occurs as oxyhemoglobin (oxy-Hb) [16]. The visible spectrum of oxy-Hb consists of 3 main peaks [26]. The strongest peak at ~ 415 nm is called the Soret band (or γ band), weaker two peaks – one at ~540 (β band) and second at 576 nm (α band). As the bloodstain ages, the spectrum changes mostly in the region of the α and β bands, because of the oxidation of oxy-Hb to methemoglobin (met-Hb) and then to hemichrome (HC) [11].

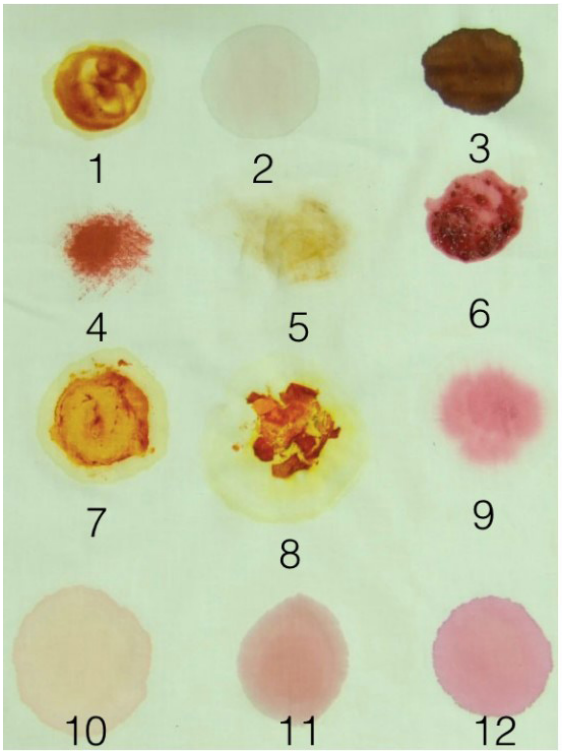
Because there has been no analysis in the literature pertaining to the use of the alternative light source (ALS) to differentiate bloodstains from simulated traces similar to blood, and due to the mentioned above properties of blood, we used the ALS as a fast and non-destructive technique to identify bloodstains from a group of traces morphologically similar to blood. The objective of our study was an experimental visualization of bloodstains in a group of stimulated bloodstains, which means that this research can help in the differentiation of blood with traces imitating blood, which can be found at the crime scene.

MATERIALS AND METHODS

The material used in the research was human blood, as well as simulated bloodstains which included: red borscht, raspberry juice, cherry liqueur, cranberry juice, tomato bruschetta, tomato paste, raspberry jam, rust, red spray, red wine and tomato ketchup (Fig.1). Blood was collected from the antecubital vein into a test tube with ethylenediamine tetraacetic acid (EDTA). Afterwards, blood and the material imitating bloodstains were applied on a sterile white cloth as a contrast backing. Prepared stains were dried at a temperature of 21°C and then visualized with the alternative light source (ALS) and CSS light emitted by the Mini-CrimeScope 400 (SPEX Forensics) with yellow, dark yellow, orange and red filters. In the ALS we used light with the following wavelength: full range spectrum of the arc lamp, UV light 300-400 nm, visible spectrum - 415, 455, 515, 535, 555 nm. The bloodstains, selected by optical methods, were confirmed qualitatively by the immunochromatographic test HemCheck-1 (Veda-Lab). We presented the results as a description and also documented them in photographs.

RESULTS

The usage of light sources without color cut-off filters does not allow for the unequivocal differentiatioin of



1 - tomato ketchup
2 - cranberry juice
3 - blood
4 - red spray
5 - rust
6 - raspberry jam
7 - tomato paste
8 - tomato bruschetta
9 - red borscht
10 - raspberry juice
11 - cherry liqueur
12 - red wine

Fig. 1. Visualization of simulated bloodstains in the daylight

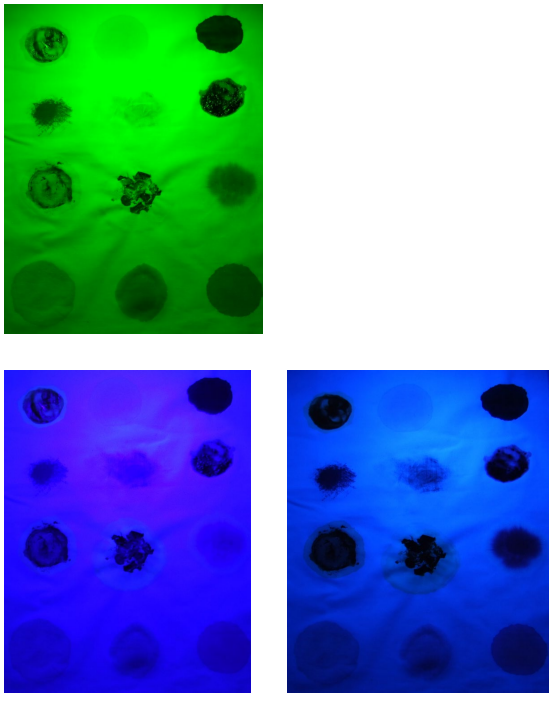


Fig. 2. Visualization of simulated bloodstains in the light with a wavelength of 555 nm, 415 nm and 300-400 nm without using color cut-off filters

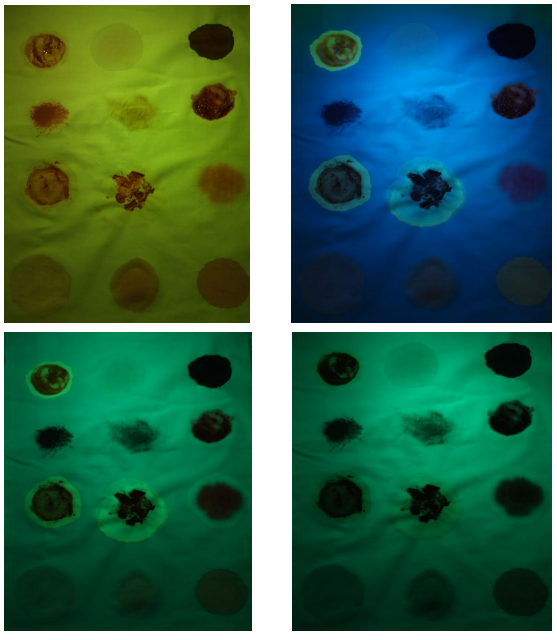


Fig. 3. Visualization of simulated bloodstains in the light with a wavelength of 555 nm, 455 nm, 415 nm and 300-400 nm using yellow cut-off filter

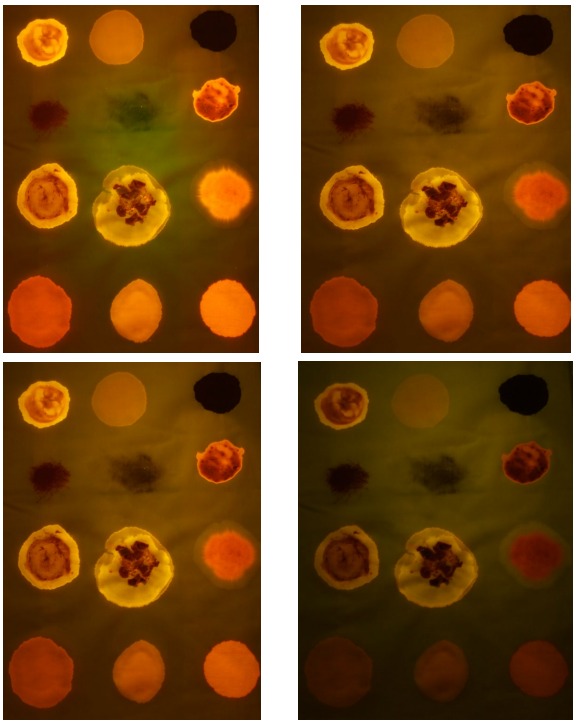


Fig. 4. Visualization of simulated bloodstains in the light with a wavelength of 515 nm, 455 nm, 415 nm and 300-400 nm using orange cut-off filter

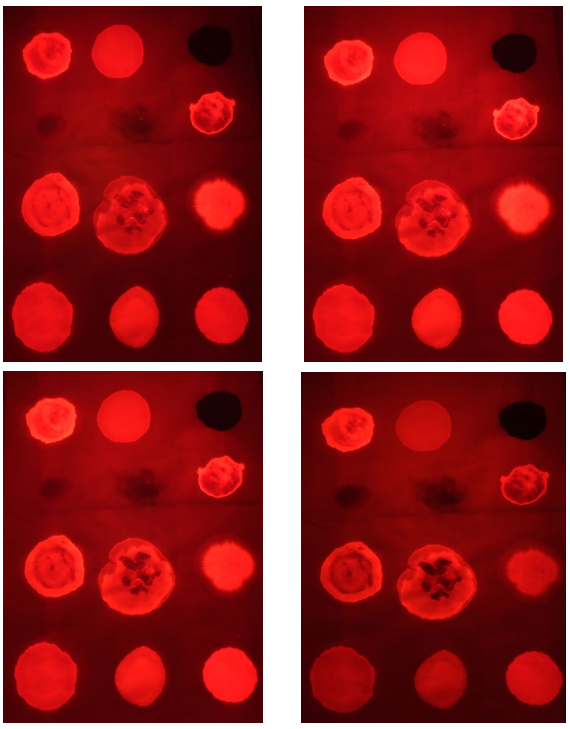


Fig. 5. Visualization of simulated bloodstains in the CSS light and the light with a wavelength of 535 nm, 515 nm and 455 nm using red cut-off filter

real bloodstains from the trace evidence imitating or resembling bloodstains (Fig. 1 and 2).

The usage of a yellow cut-off filter makes it possible to exclude unequivocally simulated bloodstains made of tomato ketchup, tomato paste and tomato bruschetta in the light with a wavelength of 455 nm, 415 nm and 300-400 nm, as well as a trace of red borscht and a trace of cranberry juice a light with a wavelength of 415 nm and 300-400 nm (Fig. 3).

The application of orange cut-off filter makes it possible to exclude unequivocally simulated bloodstains made of food and alcohol: tomato ketchup, cranberry juice, raspberry jam, tomato paste, tomato bruschetta, red borscht, raspberry juice, cherry liqueur, and red wine, using CSS light and light with a wavelength of 515 nm, 455 nm, 415 nm and 300-400 nm (Fig. 4).

The application of red cut-off filter makes it possible to exclude unequivocally simulated bloodstains made of food and alcohol by using CSS light and light with a wavelength of 535 nm, 515 nm, 455 nm, 415 nm and 300-400 nm (Fig. 5).

DISCUSSION

As the method of visualization of the ALS allows non-destructive differentiation of biological traces at the crime scene. Miranda et al. showed that a forensic expert can detect biological fluids (including blood) at a crime scene by using the ALS even several days after the crime. They proved that the fluorescence was lower when the samples were moist, while remained constant when the samples were dry, independently from the substrate on which they were deposited [13]. The results also showed that the type of surface did not influence the drying time for the different fluids and all of the fluids showed the same trend on all surfaces [13]. This method is especially helpful when the stain is on a dark background [18]. Vendenberg and van Oorschot showed that in the case of bloodstains there is a possibility to detect blood even if it has been subjected to environmental insult [20]. Of course, the light sources must be used with caution, since certain UV wavelength can damage the DNA evidence in a sample [22].

Until now, researchers have not described the practical use of the alternative light source in differentiating biological traces similar to blood. Because there are no scientific reports in this area, some basics of physics and biochemistry are necessary to understand light absorption and photoluminescence of blood and the objective of our study.

Porphyrin molecules affect differentiation of bloodstains from the group of simulated blood traces, as they are characterized by unique optical properties – the absorption of electromagnetic radiation in the visible spectrum. Porphyrin molecules are flat and their structure is based on the nuclear ring containing four nitrogen atoms (N). The porphyrins have the ability to bind metal

cations and form complexes, what conditions their specific characteristics. The most popular substances with skeletal structure of porphyrins include vitamin B12, chlorophyll, uroporphyrin, coproporphyrin and heme [9, 27]. Addition of magnesium determines the transformation of chlorin to chlorophyll (which is present in all green plants) [27]. Also bacteriochlorophyll has magnesium in its structure. Cobalt ion occurs in the molecule of vitamin B12 [2].

Heme is the basic building block of all hemoproteins within the human body (hemoglobin, myoglobin, cytochromes, catalase, peroxidase). The structure of the protein is similar, the differences are within the polypeptide chain or the valence of iron ion. Heme is a non-proteinaceous part of the hemoglobin-containing iron in its center. This is the most absorbent tissue in human organism; however, its concentration varies within the body and in some areas is very small [4, 5].

Most absorbing molecules in a mammalian tissues are: oxy- and deoxyhemoglobin, melanin, myoglobin and water. From the point of view of forensic science, the most important hemoglobin derivative is the hemochromogen, which in the presence of a reducing agent gives the two absorption bands of length of 530 and 560 nm. This test allows us to detect blood at a dilution of 1: 200 [7]. RSID-Blood immunochromatographic test (Independent Forensics) is specific for human glycophorin A. The sensitivity for RSID-Blood, used as suggested, is less than < 1 µl of human blood. When using RSID-Universal Buffer, the limit of detection (the minimal volume of blood required for a positive signal) is slightly increased from ~50 nL to ~100 nL blood [15]. On the other side, binding of oxygen by hemoglobin is associated with structural changes and changes of the shape of the particles, and at the same time it affects the differences in the absorption bands of oxyhemoglobin (oxy-Hb) and deoxyhemoglobin (deoxy-Hb) [4]. Oxyhemoglobin demonstrates the greatest absorbance at 414 nm, and weaker at 542 and 577 nm. Absorption band of deoxyhemoglobin is shifted about 30 nm and it is 433 nm and the weaker is 556 nm. There is no second weak peak as in oxyhemoglobin, while the other appears on the length of 760 nm [4, 5]. The process of absorption described above, including venous blood which contains mostly deoxyhemoglobin, in the case of our study allowed us to identify and differentiate the bloodstain from a group of simulated traces.

Despite the fact that the compounds from the group porphyrinoids are characterized by strong absorption, particularly in Soret band (all porphyrins have a strong absorption of ~ 390 - 425 nm), due to the Stokes shift most of the solutions of porphyrins and their derivatives after being exposed to UV light, demonstrate increased fluorescence. The fluorescence is also observed in the amino acids: phenylalanine, tyrosine, tryptophan; proteins: collagen and elastin; vitamins: B2 and B6 and porphyrins: hematoporphyrin (HpD) and protoporphyrin IX. A strong fluorescence occurs even in the case of trace amounts of these molecules.

This effects can be visible in our experimentally simulated bloodstains, because red borscht, raspberry juice, cherry liqueur, cranberry juice, tomato bruschetta, tomato paste, raspberry jam, rust, red spray, red wine and tomato ketchup may include substances, which show fluorescence if they are stimulated with the UV or the visible light.

CONCLUSIONS

Due to the different optical properties of blood and substances containing especially vegetable ingredients (which show fluorescence), the use of alternative light source (ALS) allows us to differentiate blood traces from a group of traces morphologically similar to the bloodstains non-destructively. Additionally, it can help to lower the costs associated with the investigation and reduce the time spent on analysis of collected evidence at the crime scene.

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