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Technical note

Analysis of the fluorescence of body fluids on different surfaces and times

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ABSTRACT

The use of screening techniques, such as an alternative light source (ALS), is important for finding biological evidence at a crime scene. The objective of this study was to evaluate whether biological fluid (blood, semen, saliva, and urine) deposited on different surfaces changes as a function of the age of the sample. Stains were illuminated with a Megamaxx™ ALS System and photographed with a Canon EOS Utility™ camera. Adobe Photoshop™ was utilized to prepare photographs for analysis, and then ImageJ™ was used to record the brightness values of pixels in the images. Data were submitted to analysis of variance using a generalized linear mixed model with two fixed effects (surface and fluid). Time was treated as a random effect (through repeated measures) with a first-order autoregressive covariance structure. Means of significant effects were compared by the Tukey test. The fluorescence of the analyzed biological material varied depending on the age of the sample. Fluorescence was lower when the samples were moist. Fluorescence remained constant when the sample was dry, up to the maximum period analyzed (60 days), independent of the substrate on which the fluid was deposited, showing the novelty of this study. Therefore, the forensic expert can detect biological fluids at the crime scene using an ALS even several days after a crime has occurred.

1. Introduction

Biological samples, such as blood, semen, saliva, and urine, are important pieces of evidence that can be found at a crime scene. Forensics has developed various ways to identify these fluids. One of the simplest tests that is used to detect most biological evidence is the alternative light source (ALS) [1,2].

The ALS is a simple, non-invasive, non-destructive method that is used to detect biological fluids [3,4], wounds (contusions, ecchymosis, bite marks) [5,6], human remains [7], and a range of other types of evidence. An area should be scanned with an ALS before the application of other reagents [8,4].

The principle behind ALS technology is based on the absorptive and photoluminescent qualities of the item under examination [6]. Fluorescence is the property of absorbing light of a lower wavelength and emitting light of a greater wavelength [2].

Some authors [2,7,9] have suggested that new research should be performed to perfect the ALS technique. However, most studies have evaluated stains only on fabric, and it is important to analyze the influence of time on detecting the stain [5].

There has been no analysis of the use of Megamaxx™ brand lights or the influence of time on the detection of stains on different surfaces. Therefore, these factors are the novelty of this study. This research can help improve the ALS technique and aid the forensic expert in collecting evidence at the crime scene. The objective of this study was to evaluate whether the fluorescence of a biological fluid deposited on different surfaces changes according to the age of the sample, when illuminated with an ALS.

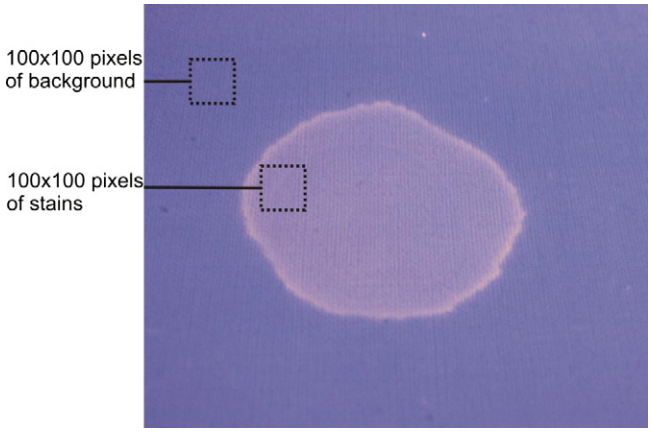

Fig. 1. Regions of interest (ROIs) selected for analysis.

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Table 1
ANOVA results for effects studied under an ALS, with the appropriate model for the experiments, using two fixed factors and time (sample age) defined through repeated measurements.

Effect	Degrees of freedom		Analysis of variance	
	Numerator	Denominator	F Statistic	p-Value
Surface	4	50	80257.5	0.0001
Fluid	4	50	61791.2	0.0001
Age	5	250	7537.6	0.0001
Fluid * surface	16	50	6836.0	0.0001
Surface * age	20	250	167.49	0.0001
Fluid * age	20	250	995.65	0.0001

Table 2
Mean brightness values with respect to the "sample age" factor in the control group for each surface.

Surface	Sample age	Tukey group ($\alpha = 0.05$)
Tile	1 min	A
	1 h	A
	24 h, 10, 35, 60 days	A
Wood	1 min	A
	1 h	A
	24 h, 10, 35, 60 days	A
Paper	1 min	A
	1 h	A
	24 h, 10, 35, 60 days	A
White fabric	1 min	A
	1 h	A
	24 h, 10, 35, 60 days	A
Black fabric	1 min	A
	1 h	A
	24 h, 10, 35, 60 days	A

2. Materials and methods

Body fluids (blood, semen, saliva, and urine) for the experiments were obtained from a volunteer donor. Samples were utilized shortly after their collection, without using preservatives, except for the intravenous blood that was collected in a tube containing ethylenediamine tetraacetic acid (EDTA). The EDTA was employed to avoid coagulation and does not interfere with blood detection [8]. The Research Ethics Committee of the Piracicaba Dental School (FOP/UNICAMP) approved the study under case no. 051/2012.

Body fluids were deposited on the following porous and nonporous surfaces: wood, black cotton fabric, white cotton fabric, paper, and white tile. The surface–fluid unit was exposed to an ALS (Megamaxx™ System; Sirchie, Youngsville, NC, USA) at 1 min, 1 h, 24 h, 10 days, 35 days, and 60 days after the fluid was deposited on the surface. These exposure times can be considered as the storage time/age of the stains, which were stored at room temperature.

Stains were illuminated with the ALS at a wavelength of 455 nm (as suggested by the manufacturer) and viewed with orange glasses. The ALS equipment was mounted on a tripod, to maintain a fixed distance between the ALS and the analyzed stain. A diffuser attached to a lamp was used to make the light softer.

Photographs were obtained with a Canon EOS 60D digital camera, using a Canon EF-S 60 mm f/2.8 Macro USM lens (Canon Inc., Tokyo, Japan) and an orange-colored lens filter (Tiffen Company, NY, USA). The camera was mounted on a tripod to avoid movement. The camera was controlled via a computer with the Canon EOS Utility™ software (Canon Inc.). The camera's ultraviolet (UV) filter was removed, to guarantee that the camera's sensor would pick up light near the UV range, as practiced by Lee [10]. Photographs were obtained in a completely darkened room.

Photoshop™ (Adobe Systems, San Jose, CA, USA) was utilized to prepare the photographs for analysis, and then ImageJ™ (National Institutes of Health, Bethesda, MD, USA) was employed to record the brightness values of pixels in the images. First, Photoshop™ was used to remove regions of interest (ROIs) measuring 100 × 100 pixels from the images. These ROIs contained the stain and background (Fig. 1). These ROIs were transformed into 8-bit/channel "grayscale" images, so that the color information could be discarded and consistency maintained during the analyses [11]. The new images were saved in .TIFF format. The ROIs were obtained in the same position in all of the tests. The surface on which the fluid was deposited (background) served as the control for the experiment. Next, the ImageJ™ software was used to obtain the average brightness value for the ROI, with the following command: "Analysis" > "Histogram". For each pixel, a numerical value was assigned, ranging from 0 (completely black pixel) to 255 (completely white pixel), which represented its brightness on the grayscale [11].

Data were submitted to analysis of variance (ANOVA), using a generalized linear mixed model with two fixed effects (surface and fluid).

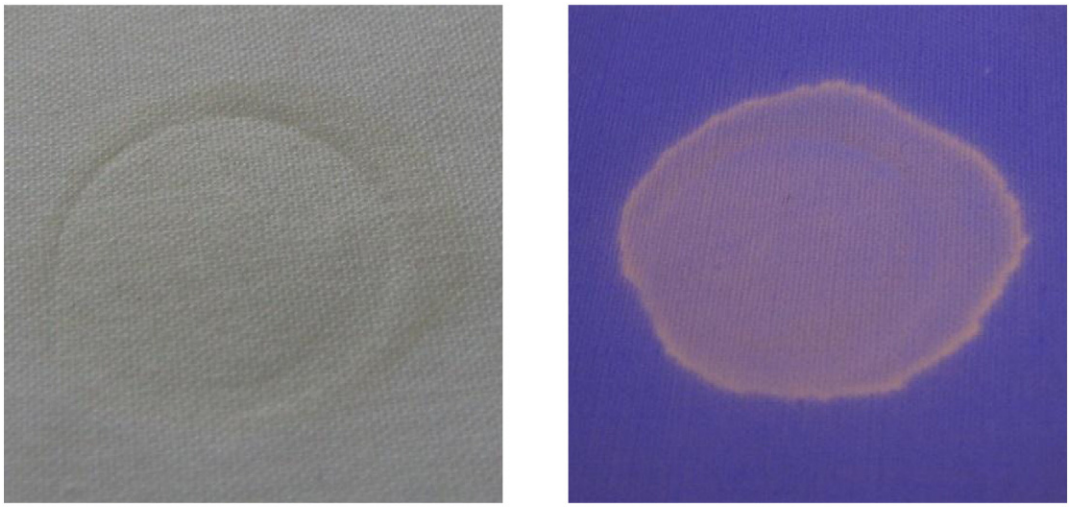

Fig. 2. (A) Semen exposed to natural light. (B) Semen exposed to the ALS.