Applying Luminol test for identification of blood traces covered under layers of soil – Uncover hidden evidences.

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ABSTRACT

The easiest piece of evidence to find at a crime scene is a bloodstain which plays a significant role in individual identification. One of the most significant and well-known tests that has been successfully used for the alleged detection of bloodstains at a crime scene that are often invisible to the human eye is the luminol test. The visibility of chemiluminescence on various soil layers is the main area of interest in this work. Visualization of CL in layers of soil was analyzed in which the chemiluminescence was visible till 4mm layer of soil i.e., from direct, 1mm, 2mm, 3mm, 4mm and 5mm soil above the blood after 4 mm the visualization without disturbing the surface were not possible. The intensity of the chemiluminescence decreases with increase in layers of soil without disturbing the surface.

Key Words: Blood, luminol, soil, chemiluminescence, layers of soil, luminol photography, presumptive blood test, blood detection.

INTRODUCTION

One of the body fluids that forensic scientists come into contact with the most is blood, especially when it comes to violent crimes. It is an excellent source of DNA, and blood pattern analysis can help determine the likelihood of both prosecution and defence scenarios.(3) Blood in animals carries nutrients and oxygen to cells as well as metabolic waste products away from those cells. Blood is made up of fluid blood plasma and cellular blood cells or corpuscles. In addition to dispersed proteins, mineral ions, glucose, carbon dioxide, hormones, platelets, and blood cells, plasma mostly consists of water. Mostly, erythrocytes, leucocytes, and platelets make up the blood cells.(1)

There are various methods used in the identification of blood. Different types of test such as preliminary test, confirmatory test, species of origin, DNA profiling are few methods used in analysis blood sample. Amongst which preliminary test is performed to analyze whether the

stain or the sample you have received is blood or not.

Preliminary tests, such as the Benzidine test, can be used to identify visible blood stains, but invisible or cleaned-off bloodstains or concealed bloodstain under layers of soil must first be detected before being recognised. To find blood stains hidden beneath several layers of soil would be a difficult task. However, a special reagent known as luminol can be employed to get around this problem of hiding blood evidence.(2) Luminol and Polylight are the methods most commonly used during crime scene examination. (1)

The (3-aminophthalhydrazide) luminol reagent combines with haemoglobin in red blood cells to produce a bluish chemiluminescence. Since Walter Sprecht's original discovery, the forensic luminol test for blood has been well-known.(7, 4, 8) Albrecht published the first research on luminol's chemiluminescent characteristics in 1928.(7)

In Luminol test hydrogen peroxide or sodium perborate, both oxidising agents, are present in a single reagent that is applied in an aqueous, alkaline state. As this substance degrades, oxygen released from the reaction between it and luminol creates the unstable 3-aminophthalate, which decays while emitting light at 454 nm.(3) Luminol is a chemiluminescent substance that can be used to chemically enhance blood on various surfaces as well as a presumptive test for blood.(2,10)
The ability of the luminol test to highlight the presence of scattered, very small droplets of blood by the individual sparkles of blue chemiluminescence each droplet produces is a special feature that makes it somewhat more useful than the other three common presumptive tests for blood (the benzidine, phenolphthalein, and leuco-malachite colour tests. (6)
Despite the fact that the test is now well-established as a blood presumptive test, it is prone to provide false positive indications of the presence of because several common and industrial compounds can catalyse chemiluminescence just as well as haemoglobin, blood can produce chemiluminescence.(11)
Chemiluminescence, which can take place in solid, liquid, or gaseous environments, is the term used to describe the emission of light from a chemical process. In recent years, numerous textbooks and articles have thoroughly explored the principles of chemiluminescence.(5,8) The majority of serological tests and DNA analyses used for identification are performed without interference when luminol is used.(9) Hence it is Said that the usage of luminol as presumptive test will not denature the DNA.

METHODOLOGY

Luminol reagent preparation:

The luminol reagent was prepared based on the quantities and reagent mentioned on the table (1). Over all 300 ml of the reagent was prepared. The solutions were kept separately in three different amber glass bottles. 30 ml of each solution were taken in a dark colored spray bottle.

Each of the solutions were mixed together by shaking and allowed the solution to rest for few minutes before it was used.

Reagents and solutions	Amount	Molarity/Normality final concentration
Sodium Hydroxide	1.6 g in 100 ml water	0.4 N
Hydrogen Peroxide	10 ml of 6% H_2O_2 in 100 ml of water	0.176 M
Luminol	0.07 g in 100 ml water	0.004 M

Table 1: The reagent, quantity of chemicals taken, final concentration in molarity/ normality has been tabulated. [1]

Application of luminol:

Luminol solution was performed in a dark room for the visualization of the chemiluminescence produced by the luminol when reacted with blood stains. Luminol was sprayed gently on the surface where blood was present. Photographs of each sample were taken with the shutter speed of two seconds. By keeping the lights on, the camera was kept in focus, and by the time luminol was sprayed on the sample, the lights had been switched off. Each sample was twice sprayed, and the camera recorded the chemiluminescence.

Sample collection:

Blood sample was collected in EDTA vials from three different individual of same blood group. 15ml of blood was collected 5ml from each individual.

Figure 1: Soil surface containing blood stain samples

Procedure:

- Soil samples were collected from a ground. It was sieved with sieve size 85
- Transparent plastic boxes were taken and on all the four side of the box's measurements up to 2 cm were drawn.
- Soil was then poured inside the box till 1 cm and the soil was levelled using a small roller.
- 50µl of blood was added on the surface of the soil in the center. Similarly, 5 boxes were made.
- After putting blood on the soil each box was filled with layers of soil from a count of 1mm, 2mm, 3mm, 4mm, 5mm.
- The photographs of the samples were taken and then samples were taken to a dark room for capturing the chemiluminescence produced by the blood in the soil.
- Luminol was put on the soil surface using droppers with measurement where blood was present. Photographs of each sample were taken with the shutter speed of two seconds. By keeping the lights on, the camera was kept in focus, and by the time luminol was put on the sample, the lights had been switched off. The camera recorded the chemiluminescence.

Figure 2: layering and sieving the soil sample using roller and sieve in plastic container with measurements

OBSERVATIONS:

The photographs of the samples before and after treating it with luminol were taken. The chemiluminescence was seen in direct blood and recorded 1ml of luminol was poured on the sample, similarly it was seen in 1mm, 2mm to which 1ml of luminol was poured. In 3mm the chemiluminescence was seen when 1.5 ml of luminol was poured. In 4mm the

chemiluminescence was produced but in a less amount when 2ml of luminol was poured. In 5mm there was no chemiluminescence seen even after pouring 5ml of luminol on it.

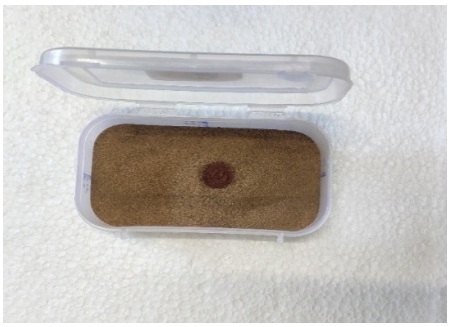

Figure 3: Blood sample directly added on the surface of soil

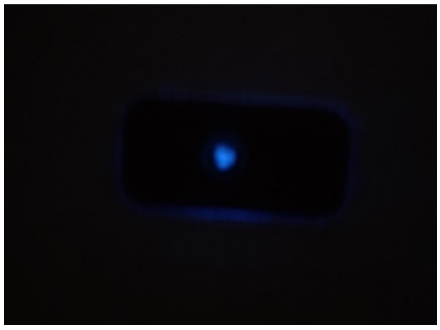

Figure 4: CL produced by the sample treated with luminol in soil surface

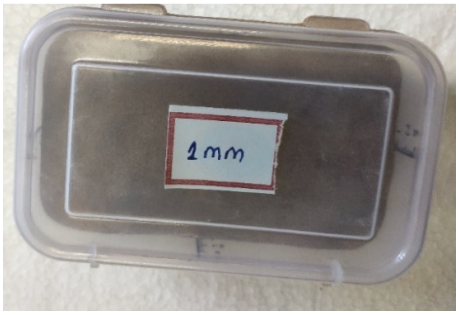

Figure 5: Blood sample to which 1mm layer of soil is added above the blood stain

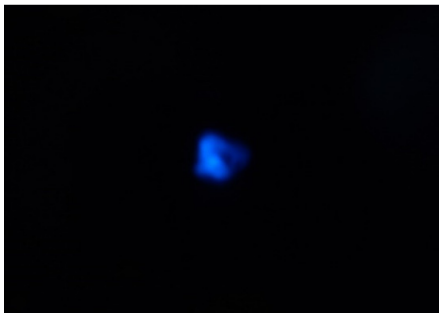

Figure 6: CL produced by the sample treated with luminol in 1mm layer of soil above the blood stain

Figure 7: Blood sample to which 2 mm layer of soil is added above the blood stain

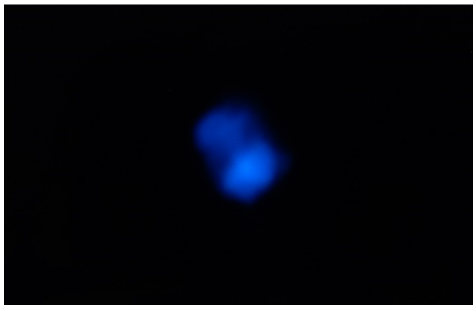

Figure 8: CL produced by the sample treated with luminol in 1mm layer of soil above the blood stain

Figure 9: Blood sample to which 3 mm layer of soil is added above the blood stain

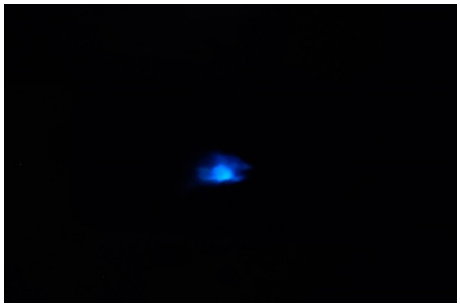

Figure 10: CL produced by the sample treated with luminol in 3 mm layer of soil above the blood stain

Figure 11: Blood sample to which 4 mm layer of soil is added above the blood stain

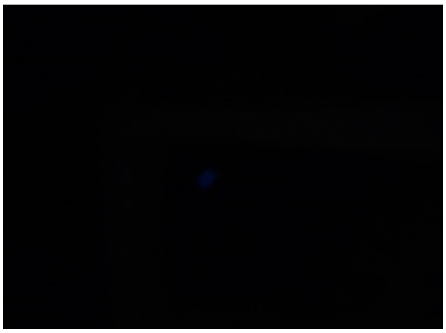

Figure 12: CL produced by the sample treated with luminol in 4 mm layer of soil above the blood stain

Figure 13: Blood sample to which 5 mm layer of soil is added above the blood stain

Figure 14: No CL produced by the sample treated with luminol in 5 mm layer of soil above the blood stain

RESULT AND DISCUSSION

Visualization of chemiluminescence in layers of soil was analyzed in which the chemiluminescence was visible till 4mm i.e., direct, 1mm, 2mm, 3mm, 4mm and 5mm soil above the blood after 4 mm the visualization without disturbing the surface was not possible.

CONCLUSION:

This research intends to observe chemiluminescence in the layers of soil that were investigated and where it was discovered that the chemiluminescence could be observed till 4mm soil above the blood after 4 mm the visualization without disturbing the surface is not possible. The research paper concludes that the chemiluminescence can be visualized on the blood stain present on the soil surface. The visualization is possible when 1mm, 2mm, 3mm and 4 mm of soil layers above the blood stain. The intensity of the chemiluminescence decreases with increase in layers of soil without disturbing the surface.

Compliance with Ethical Standards:

- Disclosure of potential conflicts of interest: N/A
- Research involving Human Participants and/or Animals: N/A
- Informed consent: N/A
- Funding: No funding
- Ethical Approval: N/A
- Data Availability Statement: N/A

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