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Evaluation of Efficiency of Luminol test on Different Substrates for Latent Blood Detection: A Review Study

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ABSTRACT

Blood has a highly great role to play in crime investigation and crime scene reconstruction as a forensic evidence. When no bloodstains are found by naked eye on crime scene investigations the luminescence produced by the luminol test for blood has a useful benefit. Luminol has been effectively utilized for more than 40 years for the presumptive detection of latent bloodstains and it is well known technique in field of forensic investigation. This test has a high responsiveness to blood, as compared to other forensic blood assays as it can identify the blood presence in scale of nanograms. However, luminescence can be also produced by other interfering compounds, leading to a misinterpretation for the presence of blood. By treating blood with luminol spray, it can also produce detrimental effect on the serological assays of blood stains. This present review paper studies the efficiency of reagent luminol with different kinds of substrates and described its advantages and disadvantages for latent blood detection.

Keywords: Blood; Luminol; Chemiluminescence; Serology; Bloodstains; Forensic; Crime Scene; Haemoglobin; Substrates.

1. INTRODUCTION

Blood evidence is frequently encountered in the forensic investigation of violent crime. Forensic analyses of the blood found at a crime scene serves valuable information that can be crucial in the solving of a crime cases [1]. However, the crime scene can be altered by the accused to conceal any evidence left behind, which can mislead the investigation. Advances in forensic science had provided some reagents to support special analysis of evidence. These reagents can disclose the traces which are invisible to naked eye. The reagents used by the experts are part of the identification tests. There are several examples like, benzidine test, which can detect the presence of hemoglobin in the sample by the indication of a blue colour. This result establishes the oxidation of the hemoglobin molecule by hydrogen peroxide present in the reaction sample. A disadvantage of this method is the DNA degradation within forty-eight hours later use. Another test, known as Kastle-Meyer, also reveals a change in medium colour in the presence of hemoglobin. It goes from colourless to pink colour due to the presence of phenolphthalein. Luminol test is a third assay and has been considered one of the most well-known test in the field of forensic science. Preliminary tests are frequently employed during the investigation of a crime scene to detect any invisible traces of blood. One of the most commonly used presumptive tests is luminol, which display chemiluminescence (in the form of a blue gleam) in the presence of hemoglobin found in blood. [2]

1.1. History of Luminol

The compound 3- aminophthalhydrazide (5-amino 2,3dihydrophthalazine 1, 4-dione) was first established in 1902(1). Early researchers noted that the compound exhibited a blue chemiluminescence in the presence of other chemicals; i.e., a blue light was produced by chemical means. In 1934, this compound was termed as LUMINOL which means producer of light (2). (1Schimtz, 2Huntress)

The first proposed forensic application of Luminol as a presumptive blood test was reported in 1937(3). Blood was sprayed on bushes, stone walls, rusty iron fences, furniture, stone steps and a garden. After allowing the blood to remain exposed to

the environment for 14 days, a luminol solution mixture was sprayed onto the blood and the results was photographed. All blood-stained areas glowed with blue light for 10 to 15 minutes. Blood was also detected in water, soapy water and sewage. The luminol test worked well with both fresh and old bloodstains, in fact, the older bloodstain, gives more apparent positive reaction. (3Specht)

In 1939, by using Luminol's spray mixtures, bloodstains were detected on paper, fabrics and iron pipes exposed to the elements for 3 years, with 3-year-old putrified blood exhibiting intense luminescence (4). It was observed that dried and decomposed blood generate a stronger and longer lasting luminol reaction than fresh blood. When the luminescence disappeared, it could be reproduced by application of fresh luminol spray. Dried bloodstains were made luminescent many times. Fresh dried bloodstains were made more luminescent by spraying the blood with 1 to 2% hydrochloric acid solution before luminol application. The luminol reaction was obtained with both animal and human blood. (4Proescher & Moody) [3]

REVIEW OF LITERATURE

- J. I. Creamer *et al*. (2003) have studied the substances that interfere with the forensic luminol test for blood. This study had been concluded the nine major commonly occurring substances that can easily mistake for blood. These are, turnips, parsnips, horseradishes, commercial bleach (NaCLO), copper metal, some furniture polishes, some enamel paints, and some interior fabric in motor vehicles.

- Ana Castello *et al*. (2008) have studied bleach interference in forensic luminol tests on porous surfaces. This studied shown household bleach interference with luminol testing disappears after a given drying time when porous surfaces are involved. It was also concluded that, when a surface is suspected of having been washed with household bleach, it is recommendable to wait for a certain amount of time before applying the reagent luminol.

- Jonathan I. Creamer *et al.* (2015) have studied the effect on the luminol test when an attempt is made to clean bloodstained tiles with a known interfering catalyst (bleach). It was concluded that the emission of chemiluminescence has been increased, when solutions of bleach have been added to luminol solution.

- DeepthiNagesh, Shayani Ghosh (2016) have studied, efficiency of luminol in the detection of bloodstains concealed by paint on different surfaces. It was concluded that the nature of the substrate containing the bloodstain, nature of paint and time since concealment greatly influenced the intensity of chemiluminescence.

- Monika Gupta, Vaibhav Saran (2016) have examined Traces of blood Stains on different fabrics after washing. There are many presumptive tests were used to detect the bloodstains on clothes after washing, but luminol test was found to be most sensitive for detection of the washed stains on clothes.

- C. Oldfield *et al.* (2017) have studied the efficacy of luminol in detecting bloodstains that have been washed with sodium percarbonate and exposed to environmental conditions. The findings of this study suggest sodium percarbonate is able to remove detectable traces of blood from denim and from carpet under certain conditions (high washing temperature and after exposure to environmental conditions). It also has been suggested that cold water is more proficient at removing bloodstains than hot water. It also given that luminol has been shown to be more effective at detecting aged bloodstains as opposed to fresh.

- Valentina Brenzini, Rahul Pathak (2018) have studied detection of bloodstains on painted and cleaned surfaces with luminol. This study investigates the effectiveness in reducing the detectability of bloodstains on ceramic tiles using four different cleaning methods pure water, soap with water, wet wipes, and bleach. It also investigate the detectability of bloodstains on painted ceramic tiles as a function of the number of layers of paint.

- Michaela Hofmann *et al.* (2018) have studied the effect of machine washing on bloodstains on both cotton and polyester cloths. It has been concluded that it is generally possible to detect blood after a machine wash, but the quality of the retained bloodstains is depending on a multitude of factors like the type of the washing machine, the washing temperature, the filling degree of the washing machine, and the drying conditions of the bloodstain (temperature, duration).

INTERFERENCES BY DIFFERENT SUBSTRATES ON LUMINOL REACTION

The luminol assay for blood has a very high receptivity for blood, which makes it as an advantageous tool in forensic. The result of luminol's assay may be changed by a broad range of interfering compounds, commonly found in home environment, such as iron salts, copper, iodine, potassium permanganate, animal hemoglobin, plant peroxidases and hypochlorite ion. The latter represents the principal reason of a false positive reaction. The clearance of a bloodstain upon washing with hypochlorite solution removes more hemoglobin compared to water alone. As the hemoglobin concentration reduces, the emission of light by luminol is reduced. However, a successive washing with hypochlorite solution or bleach promotes the accumulation of this ion which increases the extent of intensity of light emission by luminol reaction. In other words, the hemoglobin is replaced by hypochlorite ion leading to a light emission equivalent as the one emitted by a blood stain that has only been washed with water. [2]

A wide range of domestic and industrial substances that might be mistaken for hemoglobin in the forensic luminol test for blood had been detected. The substances that are previously studied were in the categories of vegetable or fruit pulps and juices; domestic and commercial oils; cleaning agents; an insecticide; and various glues, paints and varnishes. A significant number of substances in each category gave luminescence intensities that were comparable with the intensities of undiluted hemoglobin, when sprayed with the standard forensic solution containing aqueous alkaline luminol and sodium perborate. In these cases the substance could be easily mistaken for blood when the luminol test is employ, but in the remaining cases the luminescence intensity was so weak that it is unlikely that a false-positive test would be generated. The results shows that particular care should be taken to avoid interferences when a crime scene is contaminated with parsnip, turnip or horseradish, and when surfaces coated with enamel paint furniture are involved. Some care should be taken when surfaces covered with terracotta or ceramic tiles, polyurethane varnishes or jute and sisal matting are involved. [4]

Several compounds can influence the luminol test reaction sensitivity are-

Suppressors or Reducers: **The compounds which can suppress luminol emission can be classified into three classes that can be -**

a. Ligands with high affinity or reactivity for a specific oxidation state of iron E.g., Cyanide, sulphide.
b. Compounds acting as an anti-oxidizing species i.e. materials with a standard reduction potential smaller then luminol, thus preventing luminol oxidation. E.g., tannins, anilines.
c. Compounds acting as chemiluminescence filters or quenchers. [5]
d. *3.2 Provokers or Enhancers:* The compounds which can generate luminol emission can be classified into three classes that can be –
e. Compounds showing a catalytic peroxidase or peroxidase like activity such as inorganic compounds containing free metal ions. E.g., soils, minerals, rust, metal objects.
f. Compounds with a high oxidising capacity towards luminol such as organic compounds containing mild-strong oxidants. E.g., hypohalites.
g. Complex chemical composition compounds with an undefined action mechanism towards luminol mixture. E.g., oils, glues, carpets, sinks, automobile seats, paints & varnishes. [5]

CONCLUSION

The importance of luminol for forensic specialists is remarkable due to its high sensitivity and easy handling. Minute amounts of blood can be visualized through the chemiluminescence. Many factors influence the potential to detect blood. Besides its higher sensitivity, several components and substrates can trigger CL that can be mistaken as blood. When bleaches are used to clean bloodstained areas, they can interfere with the luminol test. To decline this problem a crime scene can be allowed to air for one to two days. In several scientific studies, it has been shown that use of luminol solution does not seen to have a severe inhibitory effect on neither PCR nor fluorescent STR typing. However, there is a risk of DNA-destruction that increase with prolonged exposure and the test imply a risk of contaminating the detected blood when the solutions are sprayed upon it. It should be kept in mind that the luminol test for blood frequently visualizes blood that otherwise would not have been found at all by other suitable means. Although vital importance is the understanding that luminol remains a preliminary blood screening test which alone is insufficient to conclusively establish the presence of blood. Luminol is a serologically destructive reagent. Preliminary screening tests must be employed at a crime scene on confirming suspected bloodstains, it is suggested that if blood is visible, the stain should be preserved, properly packaged and sent to a forensic laboratory for analysis without luminol testing. If no visible blood is present, one should consult with a forensic serologist and determine whether the use of luminol would be suitable or not.

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