Using Luminol to Detect Blood in Soil Eight Years after Deposition

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Abstract

In October of 2004 six testing grids were created on a hilltop at the Highlands Ranch Law Enforcement Training Facility located in Douglas County approximately eleven miles south of Denver, Colorado (USA). Each grid unit measured 24 x 24 in (61 x 61cm). The authors poured 500 ml of neat horse blood into each grid unit at the commencement of the study to test the effectiveness of using the blood reagent luminol to detect the blood pattern and presence over prolonged exposure. The testing was originally expected to last no more than 24 months. This study marks the eighth year of successful blood detection at this site using luminol.

Keywords: Luminol, blood detection, bloodstain pattern analysis, crime scene reconstruction, forensic science

Introduction

The luminol reagent (3-aminophthalhydrazide) reacts with the hemoglobin in red blood cells producing a bluish chemiluminescence. It is estimated that the sensitivity of luminol to blood is approximately 1:1 000 000 parts [1]. The use of luminol (and its derivatives) to detect diluted blood has been reported as early as 1937 and is commonly used today by forensic analysts and crime scene reconstructionists [2-3]. Luminol has been shown to be effective in detecting blood on various surfaces following exposure to precipitation, water flow, and efforts of cleanup with various chemical cleaning agents [4-8]. Quickenden, et al., [4] tested the effectiveness of luminol on various washed surfaces inside motor vehicles. An observation of note in the study was the conversion of hemoglobin to methemoglobin after exposure to increased heat in the vehicle following blood staining. This conversion produced an increased (enhanced) sensitivity of the luminol reaction. Luminol has also been used to detect blood from extremely old crime scenes. In 2004, researchers used luminol to detect blood on the floor joists below the murder site of Mr. Andrew Borden in the infamous Lizzie Borden murders of 1892 in Falls River, Massachusetts [9]. The blood was approximately 112 years old at the time of the reaction. These studies and
Experiments indicate that compounds in blood are very persistent and that luminol may be an effective reagent to detect diluted blood in a variety of environments.

**Experiment Design**

In October of 2004, the authors designed an experiment to test the effectiveness of luminol in detecting blood in soil over prolonged periods of exposure. An exposed hilltop was selected at the Highlands Ranch Law Enforcement Training Facility (HRLETF) in Douglas County, Colorado (USA). The study location is on the crest of a fully exposed hilltop within the controlled law enforcement facility. The elevation of the experiment site is approximately 6000 ft (1830 m) above sea level and is comprised of grassy meadows and rolling hills of Gambel Oak (*Quercus gambelii*) with scattered stands of conifer. Annual precipitation between October 2010 and October 2012 was approximately 35 in (90 cm). Six testing grids, each measuring 24 in (61 cm) square were laid out in a row along the crest of exposed hilltop. The authors poured 500 ml of neat horse blood in an “X” pattern inside each grid unit. Nothing was done to protect the blood from exposure to the environment. After one week of exposure, the blood pattern was no longer visible to the naked eye. During the initial 24-month experiment, each half of the “X” pattern was sprayed with the luminol reagent every two months. The other half was protected with plastic sheeting. Luminol was mixed on site using the following formula:

- 0.5 g 5-amino-2,3-dihydro-1,4-phthalalazinedione (luminol)
- 25 g sodium carbonate
- 3.5 g sodium perborate
- 500 ml distilled water

Results of these experiments have been reported in 2-year intervals [10-13]. The “X” pattern was detectible on the soil surface up to 16 months after which the surface had to be scraped to expose the blood that had drained to lower strata. The research site has been under law enforcement control since 1985 and access is restricted to a limited number of authorized personnel. No other blood experiments have been conducted on the research site. Between October 2010 and October 2012 the authors did not conduct any activity on the site aside from an occasional visual inspection.

**Results**

On the night of November 9, 2012 the authors returned to the experiment site to conduct additional testing. Photographs were taken of the grid units prior to any application of luminol (Fig. 1). The authors did not observe any disturbance, discoloration, or visible staining of the soil, which was covered with scattered patches of grass and weeds. Grid units #2 and #3 were selected for testing since neither had been tested in the previous visit two years earlier. Grid unit #3 showed some immediate surface chemiluminescence upon initial spraying but the reaction area was less than 2 in (5 cm) in size (Fig. 2). As with previous experiments, the soil did not exhibit any false positive reactions outside the testing grids. After initial luminol testing, the authors scraped away several inches of topsoil. This technique yielded successful results in previous experiments on this site.

After removing the topsoil luminol was applied again and a larger area of reaction was observed (Fig. 3). Some “streaking” was observed with the reaction area which was determined to be caused by the shovel.

![Figure 1: Testing area prior to reagent application.](image-url)
“pushing” soil containing trace amounts of blood. Luminol was applied to the shovel with negative results. The shovel was also used to scrape soil in an area adjacent to the testing grid (not known to contain blood). This scraped area was sprayed with luminol. No reaction was observed. There were no false positive reactions observed anywhere outside the testing grid. This finding is consistent with observations during previous applications of the reagent.

Discussion
The ability to detect blood in soil many years after deposition has some practical value to cold case investigations and crime scene reconstruction. Investigators may be able to locate and verify the initial body location even after being moved by suspects or animal scavengers. Locating these sites may help corroborate statements made by witnesses or co-conspirators. Additionally, these sites may contain additional evidence such as clothing fragments, cartridge cases, bullets, or other trace evidence crucial to the investigation. During the initial months following blood deposition, our findings indicate that certain bloodstain patterns such as pooling, drag marks, or patterned voids may also be discovered.

Although our results indicate that blood may be detected in soil many years after initial deposition, there are some significant limitations. DNA testing was not attempted due to the prolonged exposure. Additionally, the application of luminol is impractical over large areas. This means investigators must prioritize potential search sites. Aside from a living witness, investigators may consider the use of properly trained cadaver dogs to locate possible search areas in large open areas such as fields.

It is hoped that other investigators will initiate similar studies in their areas to better understand the limitations of luminol testing in outdoor environments. Results of such experiments will undoubtedly add to our understanding of these processes and may produce other methods of reagent application over various surfaces. We also encourage investigators to revisit old outdoor crime scenes where significant blood loss occurred. Original crime scene photographs can be used for comparison to any luminol reactions that may occur.

References


