

LUMINOL AND THE CRIME SCENE

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INTRODUCTION

To a great degree today, crime laboratories are being provided with evidence which has been treated with luminol reagent mixtures by law enforcement personnel at the crime scene. Forensic serology examiners are then requested to perform a complete analysis of the treated items for blood and other body fluids. Since the reagents are commercially available in pre-packaged crime scene kits, the luminol test is susceptible to abuse and misuse by untrained officers. In some cases, uninformed crime scene technicians opt to use luminol as a preferred field blood test. This article discusses the effect of luminol sprays on the complete serological analysis of dried bloodstains. In addition, the advantages and disadvantages of the luminol test are addressed and suggestions are tendered concerning the proper application and protocol of the luminol test at crime scenes.

THE MODERN CRIME SCENE

Imagine this crime scene. You are the primary investigating officer. The body of an elderly woman is discovered at the bottom of a long staircase in the living room of her rural home. Nothing appears to be disturbed or missing. Her body is crumpled. Her face lies in a pool of blood due to a gashing head wound. She is fully clothed with no other obvious injuries. Is it an accident? Did an old woman fall down a flight of stairs, hit her head and die from the wound? Or, is it murder? Did someone kill her and make it look like an accident?

Most of the evidence directly relating to the crime is laying in front of the officer waiting for him to recognize its significance. The construction of the prosecution's case begins with the foundation laid at the crime scene. As trained, the crime scene officer will isolate the area, photograph the scene from all angles, take appropriate measurements, dust for fingerprints, supervise the removal of the body and collect and preserve anything that might constitute physical evidence of a crime while documenting all aspects of the case for later possible presentation in court.

Collected items will be processed at a crime laboratory. This is the only way to ensure that an accurate and timely scientific analysis of the evidence will be undertaken by expert examiners. The results of the analyses will be presented later in the form of a written report and/or as oral testimony in any court related criminal proceedings. The impartial scientific approach will spotlight the facts of the case and assist in leading investigators to a logical conclusion - murder or accidental death.

To a greater degree today, crime scene personnel are taking the responsibility upon themselves to perform cursory preliminary chemical analyses in the field, outside of a laboratory setting. This sometimes results in the sabotaging of their own investigation. This slap-dash cookbook chemistry can be traced back to the first undercover narcotics arrests made by peace officers. A white powdery substance was seized. Using a pre-packaged chemical test kit, the officers would mix some of the powder with the chemical reagents and look for a change in the color of the chemicals. Depending on the color change, they may have been able to conclude that the powder was an illegal controlled substance.

Police stories on television emphasized the existence of those test kits. Their reliability was upheld in the court system and police were able to arrest drug dealers on the spot with sufficient probable cause from the chemical test results. Of course, before the case came to trial, a crime laboratory was able to provide the court with a more detailed report of the identity of the substance. The packaged test kits were quick and easy to perform. Information garnered from the kits was even used as probable cause to conduct legal searches. In addition, these tests brought out that repressed part of every police officer who enjoyed fooling around with a chemistry set as a child or who secretly admired and was fascinated by the fictional detective Dick Tracy and his wrist radio or Batman and his utility belt. When science works, it is great fun but when science helps you to do your job better, it can be irresistible.

Scientific product distributors correctly recognized that police departments all over the world would clamor to purchase the newest and most comprehensive testing kits available on any range of topics, regardless of price. There are numerous kits available for the field testing of specific drugs such as cocaine, heroin, barbiturates, amphetamines and marijuana. These companies also expanded into the marketing of crime scene kits, which proved to be great time savers for police. The crime scene kits include special paper for drawing and documenting crime scenes and recording measurements. They include dusting powders, cyanoacrylate (superglue) fumers, brushes and lifting tape for fingerprint collection and preservation at the crime scene. The kits also carry special evidence collection envelopes, small boxes, plastic bags, scissors, tweezers, tape, scalpels, flashlights, tape measures, markers and other tools essential to crime scene personnel.

Problems began when these same companies also marketed chemical blood testing kits for crime scene use. Though the chemical kits were legitimate tools for forensic serology, they were being utilized by police officers who were not trained in the correct application of the chemical reagents. These tests were also misused by crime scene personnel, sometimes to the detriment of the case under investigation.

BLOOD SCREENING TESTS

The blood testing field kits currently available without exception are preliminary screening tests for the possible presence of blood. In forensic serology, the identification of blood is minimally a two-test procedure. The first test employed is a sensitive chemical screening test, which is easy to perform in any setting. The test usually consists of two separate chemical reagents, cotton swabs, distilled water and dropper bottles. The suspected stain is rubbed with a distilled water-dampened clean cotton swab. One drop of one chemical is added to the swab followed by the addition of one drop of a different chemical. If the swab changes color within about 10 seconds then the test result is positive. No color change indicates a negative result.¹

The phenolphthalein preliminary screening test² is routinely employed by the FBI Crime Laboratory in Washington, D. C.

¹GAENSSLEN, R. (1983). Sourcebook in Forensic Serology, Immunology and Biochemistry. United States Department of Justice, Washington, D.C.

²KASTLE, J.H. (1909). Chemical Tests for Blood. United States Hygienic Laboratory Bulletin 51. United States Public Health and Marine Hospital Service, U.S. Government Printing Service, Washington, D.C.

Historically known as the Kastle - Meyer test³ with a long tradition in forensic serology dating back to 1901, it is very sensitive, quick and easy to perform. It can detect blood in dilutions of one part per 1,000. The reaction time for the test is three seconds or less. The two chemical reagents employed are a reduced, colorless chemical in a base solution called phenolphthalin and a 3% solution of hydrogen peroxide. The phenolphthalin is easily manufactured in a laboratory but must be stored refrigerated in a darkened bottle with zinc metal shavings in order to keep it in a reactive reduced state. If phenolphthalin is reacted with hydrogen peroxide and blood on a dampened swab, it will oxidize to a pink-colored chemical called phenolphthalein. The color change on the swab from clear to pink-red is indicative of a positive result. This preliminary screening test is also commercially available in a crime scene kit.

Preliminary screening tests are more important forensically for their negative results than for their positive results. If the test is negative, then the examiner knows that either no blood is present on the item or blood may be present but is in a condition not lending itself to further characterization. It is usually an extremely sensitive test which in many cases is able to detect blood in concentrations not visible to the human eye. Unfortunately, these tests give positive results for substances other than blood. They are sensitive but not specific for blood. Therefore, a preliminary test alone is not sufficient to conclusively determine the presence of blood. In effect, this test screens out any items which do not have sufficient blood to analyze further.

After obtaining a positive result from a preliminary screening test, serologists will frequently turn to a second test to confirm the definite presence of blood. This test consists of the application of chemical reagents on a small piece of the presumed bloodstain. The reacted stain is then observed under a microscope. If blood is present, small reddish-brown crystals appear microscopically. If no blood is present, no crystals will appear. These confirmatory tests are not as sensitive as preliminary screening tests, however, they are extremely specific for only blood. If a preliminary screening test and a confirmatory test give positive results on a dried stain, then a serologist can conclude that blood is present.⁴

The confirmatory test is usually performed in a laboratory because it involves the use of a microscope and is not easily

³KASTLE, J. and SHEDD, O. (1901). Phenolphthalein as a reagent for the oxidizing ferments, Am. Chem. J. 26:526-539.

⁴Supra note 1.

rendered in a field crime scene setting. In contrast, the preliminary screening tests by their nature can be easily performed at the crime scene. Many kits exist which include different forms of the basic preliminary screening test. Some tests employ a different color change in the swab by using different reagents. For example, the leucomalachite green test reagent will turn a clear swab a green color in the presence of blood and the tetramethyl benzidine test reagent will turn a swab blue in the presence of blood. The most unusual preliminary test available in kits is the luminol test.

HISTORY OF LUMINOL

The compound 3 aminophthalhydrazide (5-amino 2,3-dihydrophthalazine 1,4-dione) was first synthesized by Schmitz in 1902.⁵ 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, Huntress⁶ called this compound LUMINOL which means producer of light.

The first proposed forensic use of luminol as a preliminary blood test was reported by Specht in 1937.⁷ He sprayed blood on bushes, stone walls, rusty iron fences, furniture, stone steps and a garden. After allowing the blood to remain exposed to the elements for 14 days, Specht sprayed a luminol reagent mixture onto the blood and photographed the results. 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 the bloodstain, the more pronounced the positive reaction.

Proescher and Moody⁸ confirmed Specht's findings in 1939 using Specht's spray mixtures. They detected bloodstains on paper, fabrics and iron pipes exposed to the elements for 3 years, with 3-year-old putrified blood exhibiting brilliant luminescence. In addition, they observed that dried and decomposed blood elicited a stronger and longer lasting luminol reaction than fresh blood.

⁵SCHMITZ, A. (1902). Uber das hydrazid der trimensinaure und der hemimellitsaure, Inaug. dissertation Heidelberg cited in Curtius T. and Semper A. (1913) Ber. Btsch. Chem. Ges. 46:1162.

⁶HUNTRESS, E., STANLEY, L. and PARKER, A. (1934). The preparation of 3 aminophthalhydrazide for use in the demonstration of chemiluminescence, J. Am. Chem. Soc. 56:241-242.

⁷SPECHT, W. (1937). Die chemiluminescenz des Hamins ein hilfsmittel zur auffindung und erkennung forensisch wichtiger blutspuren, Angew. Chemie 50:155-157.

⁸PROESCHER, F. and MOODY, A.M. (1939). Detection of blood by means of chemiluminescence, J. Lab. Clin. Med. 24:1183-1189.

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 elicited with both animal and human blood.

The luminol test is known to be extremely sensitive to the presence of blood. In 1986, Thornton et.al.⁹ stated that the unaided eye could detect blue chemiluminescence of luminol in blood diluted 1:10,000. With the use of an infrared starlight scope (night vision goggles), blue chemiluminescence of luminol could be detected in blood dilutions of 1:1,000,000.

In the luminol literature,^{10 11 12} researchers have reported false positive results (i.e., chemiluminescence of luminol in the absence of blood) from alkaline luminol in the presence of iron and copper. False negative results were obtained with luminol sprayed on cadmium-coated materials bearing blood. Cadmium has been employed as a corrosion resistant coating for materials.

LUMINOL IN CRIME SCENE KITS

The luminol test is currently marketed in a three piece crime scene kit. The first component is a glass ampule containing a mixture of luminol reagent in an alkaline solution, usually of sodium carbonate. The second component is a glass ampule of a hydroxide ion source usually a 3% solution of hydrogen peroxide or sodium perborate. The third component is a spray aerosol apparatus which resembles a small atomizer or deodorant sprayer. The commercial test kits instruct the user to pour the two ampules of chemicals into the sprayer and spray the mixture over the suspected blood stain in darkness. If the treated area emits a blue light then blood may be present on the item and it should be submitted for further serological testing. No glow is a negative result which indicates that either blood is not present on the item or it is present in such dilute or degraded condition as to preclude any further serological analyses.

⁹THORNTON, J.I., GUARINO, K., RIOS, F.G. and CASHMAN, P.J. (1986). Enhancement of the luminol test by means of light amplification, J. Foren. Sci. 31:254-257.

¹⁰ROSWELL, D.F. and WHITE, E.H. (1978). The chemiluminescence of luminol and related hydrazides, Methods Enzymol. 57:409-499.

¹¹THORNTON, J.I. and MALONEY, R.S. (1985). The chemistry of the luminol reaction - Where to from here?, CAC Newsletter, September, pp.9-17.

¹²GUNDERMANN, K.D. (1965). Chemiluminescence in organic compounds, Angew. Chemie (international edition) 4:566-573.

EFFECT OF LUMINOL ON SEROLOGICAL ANALYSIS OF BLOODSTAINS

Forensic serology is best defined as the science involving the identification and characterization of blood and other body fluids, usually found in dried stain form, on items of physical evidence relating to matters of criminal justice.

Initially, a serologist will screen an item for the possible presence of blood by obtaining a positive result from a preliminary chemical test as described above. The examiner will then attempt to confirm the presence of blood by performing a confirmatory test in the laboratory. Portions of the bloodstain would then be extracted and analyzed in the laboratory to determine whether the blood is of human origin. If human blood is identified on an item, attempts may be made to determine whether the blood is of group A,B,AB or O. Then, depending upon the size of the dried stain, it may be analyzed using electrophoresis to determine as many genetic marker protein types as possible. One such marker found in blood is the phosphoglucomutase-1 (PGM) enzyme system which may be used to separate the general population into ten different subtypes. In addition to the PGM system, other such groups found in bloodstains are haptoglobin (Hp) with three types, group specific component (Gc) with six major subtypes, erythrocyte acid phosphatase (EAP) with six types and esterase D (EsD) with six types.

In order for any of this alphabet soup to make sense, known blood samples from both the victim and any subjects developed in the investigation must be submitted to the laboratory for analysis and comparison purposes. These known blood samples may be grouped as to ABO blood type and the above-mentioned genetic markers. Once all of the scientific information is assembled, the serology examiner may be able to make expert conclusions from the findings. Forensic serology is a comparison science. If all of the information from the analysis of the questioned samples is identical to that obtained from the known blood sample from the victim, then the serologist can conclude that the victim was a possible source of the deposited blood. If, however, one element of the profile differs, then the serologist may be able to absolutely exclude the victim as a source of the bloodstain. Without known standards from the victim, suspect(s) and any other involved parties for comparison, the effective serological analysis of items of evidence from the crime scene will be rendered meaningless.

In an effort to determine the effect of luminol on the performance of these standard serological tests, a series of experiments were conducted at the FBI Laboratory.¹³ Undiluted dried human bloodstains created on fabric, paper and glass materials were sprayed with mixtures of the luminol reagent and

¹³GRISPINO, R.R.J. (1990). The effect of luminol on the serological analysis of dried human bloodstains, Crime Laboratory Digest Vol 17, No. 1:13-23.

completely analyzed using the serology protocol described above. Duplicate sets of these stains were also stored for two months at room temperature to simulate the aging of forensic samples. These stains were then sprayed with luminol and subjected to the same serological protocols as the luminol-treated fresh bloodstains.

Spraying the fresh and aged bloodstains with luminol had no observable effect on the serologist's ability to confirm the presence of human blood in the laboratory. In addition, the luminol spray had little effect on the ABO blood group determination of the stains. The major problem with luminol was noted after attempting to determine the genetic marker profile of the treated stains.

Fresh and aged bloodstains that were treated with luminol were also analyzed for the presence of seven polymorphic blood enzymes and two serum proteins. These genetic markers were easily characterized in untreated fresh and aged bloodstains. The results of the study showed that luminol routinely inactivated most blood enzymes after a short exposure in undiluted bloodstains. Only one forensic enzyme (haptoglobin) could be determined in fresh luminol-treated samples. In aged luminol-treated bloodstains, all of the assayed blood proteins were either totally inactivated or untypeable. The ability to determine as complete a biochemical profile as possible on a blood sample, the ultimate goal of the forensic serologist, is severely compromised when bloodstains are sprayed with luminol.

Historically, luminol was meant to be used sparingly as a preliminary blood screening test when there was reason to believe that blood may have been present, yet was not visible. The luminol spray regimen is appropriate for a determination of invisible blood traces on large areas such as carpets, walls, flooring or the carpeted interior of a vehicle, when no blood is obvious. In these cases, if blood is present, it is there in such low concentrations as to usually preclude further ABO or enzyme analysis. Thus, nothing is lost or compromised by luminol spray application. What is gained is the ability to screen a large item or area quickly, easily and efficiently for the possible presence of blood. Luminol spray application may develop a stain pattern which could be of interest to investigators or could suggest a mechanism by which the crime took place.

LUMINOL PROTOCOL TIPS

The typical application of luminol is as an aerosol spray. Alkaline luminol stored in an amber-darkened container maintains a relatively long shelf life. When alkaline luminol is mixed with hydrogen peroxide, the shelf life of the mixture is approximately one hour.

Luminol can be applied via direct spray or indirect swab/dropper bottle method with no loss in sensitivity to blood. If the swab regimen is implemented, it should duplicate that of the phenolphthalein test (that is, a dampened swab is touched to the suspected stain and the swab is then tested with luminol). Any swabbed area which results in a chemiluminescent glow should be well marked and preserved for future analysis using standard techniques.

If the spray regimen is implemented, the following suggestions are offered:

- 1) The luminol mixture should be sprayed in a darkened, well-ventilated room. Extended luminol exposure has been shown to be moderately toxic to the liver and kidneys;¹⁴ therefore human exposure to the spray should be limited.
- 2) Known blood and a copper penny should be included as positive controls when using the luminol spray as indicators of the success and degree of relative intensity of the chemiluminescence reaction.
- 3) False positive results may be obtained with luminol. A metal staple or carpet tack in a rug or a rusted metal vehicle interior will glow after treatment with luminol, simulating a positive blood reaction.
- 4) A camera should be available to immediately photograph any observed chemiluminescence. Respraying with luminol will restore any faded glow.¹⁵

¹⁴ SCHNEIDER, H.W. (1970). A new long lasting luminol chemiluminescent cold light, J. Chem. Educ. 47:519-522.

¹⁵ THORNTON, J.I. and MURDOCK, J.E. (1966). Photography of the luminol reaction in crime scenes, Criminologist 10:15-19.

CONCLUSIONS

If used prudently, luminol may prove to be useful in a case. Recall the crime scene involving the elderly woman at the foot of the stairs. Suppose that in the upstairs bathroom you observe that parts of the floor and wall are wet and cleaner than the rest of the bathroom as if someone hurriedly washed part of the room. No obvious blood is visible. In this case the use of luminol would be appropriate.

In the fictional crime scene, after luminol application, you may find glowing areas indicative of the possible presence of blood. You may find a glowing plastic hair dryer in the sink or a glowing spatter pattern on the wall or glowing footsteps leading out of the bathroom. These patterns can be brought out again by luminol re-application. If the spray regimen is used, glowing areas should be photographed immediately as evidence. In this example the luminol results would seem to indicate that the woman was murdered in the bathroom by someone who possibly hit her with the hair dryer and threw her down the stairs to make it appear as if she accidentally tripped and fell to her death. The murderer then cleaned up the crime scene of any traces of visible blood.

Of paramount importance is the understanding that luminol remains a preliminary blood screening test which alone is insufficient to conclusively establish the presence of blood. The appropriate use of luminol at a crime scene should be discussed and evaluated on a case-by-case basis. Luminol is a serologically destructive reagent. If preliminary screening tests must be employed at a crime scene on probative suspected bloodstains, it is suggested that if blood is visible, the stain should be preserved, appropriately packaged and sent to a forensic laboratory for analysis without luminol testing. If no visible blood is present, consult with a forensic serologist and determine whether the use of luminol would be appropriate.