

Luminol-What's Glowing On?

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Luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) is a presumptive test for detecting hidden blood; namely, that which is not visible to the naked eye. The key word is "presumptive", since there are some substances that will chemiluminesce when luminol is utilized at a crime scene.

Luminol was first synthesized in 1902.(1) The chemiluminescent properties were discovered in 1928. In 1936, crystalline hemin was shown to produce an intense reaction. The initial use for medico-legal blood identification was in 1937, although in a presumptive role. (2) Our laboratory started using luminol in 1986. With experience, luminol has been a great investigative tool in determining what events may have occurred at crime scenes. In one case, a death originally thought to have been by natural causes was proven to be a homicide. Determinations as to the original site of the victim's assault have been made. Locations containing high velocity blood spatters are more easily discovered. Attempts at cleaning up bloody surfaces are more easily uncovered. Experimentation before use at a crime scene is of the utmost importance. One must "play" with many items and substances to realize just what one is "seeing" when using luminol.

Two experiments were conducted using luminol. The first deals with the effect upon various items and chemicals routinely encountered around homes and automobiles. Just what could we expect to glow? How will these possible false positives affect our interpretations? Luminol (0.1 gram) and sodium bicarbonate (0.5 gram) are mixed together in 50 ml of distilled water and stored in a non-metallic container. This is called Solution A. Sodium perborate tetrahydrate* (1.4 gram/ 50 ml of distilled water) is likewise stored in a similar container and is called Solution B. To prepare for spraying, equal portions of solutions A and B are mixed in a CO₂-propelled thin-layer chromatography mister. This sprayer was chosen for two reasons: one) the spray is delivered in a fine mist to prevent overspraying...two) there are no metal components to come in contact with the mixed solution. To insure that the mixed solutions are not contaminated, pump-type sprayers containing metal internal workings must be avoided. Remember, luminol reacts with the central iron group in blood; therefore, it will react with other metals. Once the solutions are mixed, one has approximately one to one-and-one-half hours of working time. It is advisable to mix solutions A and B in small quantities when using luminol.

We tested the following items:

concentrated dishwashing detergent
concentrated Comet Cleanser
pennies
Drano
bleach
double "00" buckshot
Spic and Span cleanser
hair spray
carpet deodorizer
Soft-Sense hand lotion
foaming cleanser
flea spray
Arm & Hammer laundry detergent
Dawn dishwashing liquid
Armor All cleaner
Armor All protectant
Go-Jo hand cleaner
Cutter insect repellent
Vaseline petroleum jelly
spray starch
Lysol spray
Crest toothpaste
WD-40 lubricant
hydraulic fluid
3M Scotchgard
Turtle Wax polish
Raid Ant & Roach killer
Dreft baby laundry soap
Kingsford odorless charcoal starter
Conoco premium leaded gasoline
Conoco unleaded gasoline
Zippo lighter fluid

Of this list, only the first six items gave any reaction when sprayed with luminol. It was observed that the intensity, duration, and color of glow associated with these false positives did not approximate that which is normally associated with dilute blood stains. Also, pattern recognition plays an important role. At an actual scene, if one "sees" a glowing hand or footprint it is easy to interpret what originally made it. Drag-trails from one room to another easily tells where and how the victim was transported. Glowing sinks often reveal where the suspect cleaned his hands. Glowing spots on the suspect's clothes leads one to search for blood spatters. Only by experimentation, can one ascertain when the observed reaction meets one's criteria for blood or a "blood-like" substance. Even though luminol is a presumptive test for blood, it has built-in "quality-control" features. Other chemical methods can be used in conjunction with luminol. If the alternate method is presumptive for blood, one still cannot call it "blood". Only a confirmatory test will

identify blood. This list of products and chemicals is by no means an exhaustive study.

The second experiment deals with the ability of luminol to withstand time and temperature. Questions were raised as to the "traveling" capability of the separated solutions. Can luminol survive the higher temperatures associated with the interior compartments and trunks of crime scene vehicles? How stable are the solutions once the dry components are mixed with the distilled water? Is it necessary to make these solutions daily? This experiment was performed by two individuals setting up the mixtures and conditions independently. Duplicates of solutions A and B were stored separately at -18, 4, 37 and 60 degrees Centigrade for 1, 3, 5, 7, 14, 21 and 28 day intervals. Solutions A and B were mixed just before spraying on known dilute blood samples in a darkened environment to observe the chemiluminesce.

The following table grades the observed reaction from poor (P), to fair (F), to good (G) to excellent (E). These grades are arbitrarily given by this author from many past experiences in using luminol on these type of samples based on intensity, duration, and color of glow:

	day 1	3	5	7	14	21	28
temp							
-18	E	E	E	E	E	G	G
4	E	E	E	E	G	G	G
37	E	E	G	G	G	G	F
60	E	E	G	G	G	F	P

As one can interpret, luminol is somewhat stable at elevated temperatures. A cloudiness was observed to form in the separated solutions, but apparently this has no effect on luminol's ability to perform due to long term storage. This author was very surprised that any reaction had occurred at 60 degrees storage at 28 days. The cooler the temperature, the more intense the reaction with known blood. The best method to travel with luminol is to keep the solutions separate and in an insulated cooler. Our laboratory routinely uses month-old luminol which has been kept in a refrigerated environment.

Most importantly, one establishes a thorough working knowledge through experimentation and application of luminol. The old adage, "practice makes perfect", cannot be overemphasized. Experience provides the investigator with the knowledge of when and how much to spray, and what patterns can be deduced from what one is observing. Luminol enables us to walk in the footsteps of a suspect but only after blood has been shed.

*one can substitute sodium perborate (0.7 gram/ 50 ml of distilled water)

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- (1) The Chemistry of the Luminol Reaction
-- Where to From Here
J. I. Thornton, D.Crim., & R. S. Maloney, B.S.
California Asso. of Criminalists
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- (2) Sourcebook in Forensic Serology, Immunology and
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EDITOR'S NOTE: With reference to the possible reaction of Luminol with the internal metal parts of sprayers and metals in general, Luminol reacts only weakly, if at all, with most metals and their salts. The one exception is copper, which is reactive with Luminol. Our laboratory routinely uses large tank sprayers with internal metal parts for processing large crime scenes and we have encountered no problems.