J Forensic Sci, Oct. 1973, Vol. 18, No. 4

R. A. Zweidinger,[1] *Ph.D., L. T. Lytle,*[2] *M.S., and C. G. Pitt,*[1] *Ph.D.*

Photography of Bloodstains Visualized by Luminol

The chemical tests for blood currently in use depend upon the detection of hemoglobin or one of its derivatives [*1*]. Hemoglobin has several properties which make it suitable for this purpose. The most important, due to its sensitivity, is the peroxidase activity of hemoglobin heme which forms the basis of the benzidine, leuco-malachite green, phenolphthalein, and luminol tests for blood. The luminol test, which is based on the visual observation of chemiluminescence, is particularly useful forensically because of its sensitivity; as a spray reagent, it also permits the detection and observation of the shape and fine structure of the bloodstains which would otherwise be invisible to the naked eye [*2–5*].

While it is obviously desirable to be able to obtain a permanent record of luminol visualized bloodstains for court evidence, photography has previously been of little value because of the low intensity and short duration of the luminescence. However, recent advances in high speed film have justified a reappraisal, and here we describe experimental conditions which have been successfully employed to obtain a photographic record.

Modifications of the Luminol Test

Prior to photographic studies it was necessary to determine the optimum experimental conditions of the luminol test, in terms of intensity and duration of light emission. The standard luminol spray reagent [*1*] was taken as a point of reference for evaluating other formulations. The standard reagent contains a 0.1 g luminol, 5.0 g sodium carbonate, 100 ml distilled water, and 0.7 g sodium perborate. The latter was added just prior to use. Spraying bloodstains with this solution results in intense chemiluminescence due to catalysis by porphyrin bound iron. This test is capable of detecting as little as 1 ng of iron.

Variation of the solvent was first evaluated, for it has been reported [*2,3*] that the substitution of dimethylsulfoxide for water produces an increase in both the intensity and time of the chemiluminescence produced by air oxidation of luminol. Unfortunately this particular modification is not applicable to the visualization of blood, because the modified reagent is too rapidly oxidized by atmospheric oxygen even in the absence of blood heme. When sprayed on a surface, very bright luminescence is observed in all areas; this luminescence decreases uniformly with time, and no contrast with blood treated areas is apparent.

Received for publication 5 Feb. 1973; revised manuscript received 11 April 1973; accepted for publication 17 April 1973.

[1] Chemistry and Life Sciences Division, Research Triangle Institute, Research Triangle Park, North Carolina.

[2] Chemistry Department, Duke University, Durham, North Carolina.

When 95 percent ethanol is used as a solvent system, the results are more satisfactory. Since alkali metal carbonates are not sufficiently soluble in ethanol, the necessary basic conditions may be provided by making the solution 0.02 *M* in KOH. Hydrogen peroxide as the oxidant produces a very bright short lived (approximately 3 s) luminescence. Sodium perborate, although only sparingly soluble in 95 percent ethanol, still produces a bright luminescence of several min duration. The background for this reagent is lower than the standard aqueous reagent.

Several oxidants which are more soluble in ethanol than sodium perborate have been tested. A brown nonluminescent, nonfluorescent product is formed when *m*-chloroperbenzoic acid is added to a saturated solution of luminol in 0.02 *M* ethanolic KOH. No luminescence is observed when *tert*-butyl peracetate and sodium perchlorate are substituted for sodium perborate. Hydrogen peroxide and *tert*-butyl hydrogen peroxide react to give a positive test but the duration of the chemiluminescence is so short (less than 3 s) that their use is not practical. Tetramethylammonium perborate, which is very soluble in ethanol, was evaluated and found to give a very long luminescence of very low intensity.

When hydrogen peroxide is substituted for sodium perborate in the standard aqueous luminol reagent a very intense short lived luminescence is obtained with blood. Photometric measurements of these two aqueous systems in solution indicate similar total quantum yields but the duration is different. The hydrogen peroxide reagent returns to the background level in 5 s. The sodium perborate reagent has a lower maximum intensity but the luminescence peak tails for more than 30 s. It should be borne in mind that these measurements were made in solution and may not be an accurate model for these reactions on solid surfaces. The hydrogen peroxide reagent may offer an advantage over the sodium perborate reagent for photography (vide infra) because all of the luminescence is emitted in a shorter time. This increases the ratio of chemiluminescence light to ambient light for any short interval of time near the initiation of the reaction.

A compound, benzo(ghi)-perylene-1,2-dicarboxylic acid hydrazide, has been reported [*4*] to have a chemiluminescence quantum efficiency six times that of luminol. This compound was therefore compared with luminol in 0.02 *M* alcoholic KOH, using perborate as the oxidant. Although sensitive to blood, its solubility is sufficiently lower than luminol to yield no practical gain in intensity.

Schneider [*3*] has reported that the light yield of luminol chemiluminesence in dimethylsulfoxide is markedly increased by the addition of a small amount of fluorescein. Only a marginal increase is observed with the standard aqueous reagent.

Photography of Luminol Chemiluminescence

For a test sample, 50 mm × 75 mm glass slides were marked with 1:1000 aqueous dilution of blood. Filter paper strips were also moistened with this solution to test the effect of a fibrous surface. A Nikkormat FTN SLR camera with a f/1.4 (50 mm) lens was used with the Kodak 2475 High Speed Recording film, while a Nikon F SLR camera with the same lens was used with the Kodak Tri-X Panchromatic film. The 2475 film was developed in DK50 developer at ASA 4000, and the Tri-X was raised to ASA 2400 by developing with Diafine developer.

The 2475 film was evaluated first, using both the hydrogen peroxide and perborate reagent sprays. All exposures were made in a darkroom at f/1.4. Exposure times for each reagent on filter paper was varied from 6 min to 1 s, but even after a 1 s exposure a clear image was obtained with both reagents. Exposure times for photographs of the glass slides varied from 2.5 min to 1 s. All exposures gave a clear image; however, the brightness of the image seemed more dependent on the amount of reagent sprayed and perhaps the

actual amount of blood present than on exposure time. For example, with the hydrogen peroxide spray a 30 s exposure was more intense than a 60 s exposure, and a 1 s exposure was approximately equal to the intensity of a 15 s exposure. An enlarged photograph on a glass slide sprayed with the perborate reagent, 1 s exposure, is shown in Fig. 1.

The Tri-X film gave essentially the same results as the 2475 film under darkroom conditions, a clear photo being obtained even at f/2.0, 1 s exposure. In order to simulate field conditions several exposures were taken in a partially darkened room. The experiment was performed at night, 10 ft from a large undraped window. There was enough outside light (artificial) that the stopwatch could be read near the window. Under these conditions the 1 s exposure duplicated the contrast of luminescence to background obtained in the darkroom. As the exposure times were increased the background became more visible in the photographs. However, even at the longest exposure time (1 min) the luminescence was clearly visible in the photograph against a brown paper towel background (see Fig. 2). The luminescence could be seen even if it were on a white surface such as that in the upper right of Fig. 2. Although shorter exposure times give greater contrast between luminescence and background, the contrast in an overexposed photograph such as Fig. 2 may be substantially improved by printing techniques.

An evaluation of the effect of camera distance on the density of the image on the film was made by photographing a cylinder of luminol reagent at distances of 1.5, 3, and 6 ft. No difference in density of the film darkening could be distinguished, the only change being the image size and loss of resolution due to film grain size.

Samples were submitted to this laboratory by the North Carolina State Bureau of Investigation as representative of a range of surfaces and materials encountered in the field.

Figures 3, 4, and 5 are typical examples of these samples. Figure 3 is a handprint made with diluted blood on a glass plate; Fig. 4 is a bloodstained piece of linoleum flooring which had been washed until no trace was visible to the eye; and Fig. 5 is a brick which

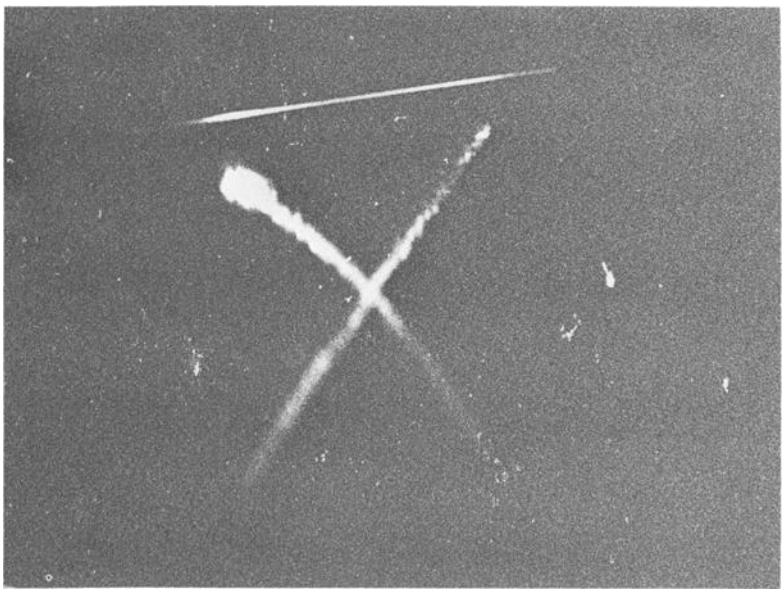

FIG. 1—*Tri-X Panchromatic film, f/1.4, 1 s. Diluted bloodstained "x" on 50 times 75 mm glass slide.*

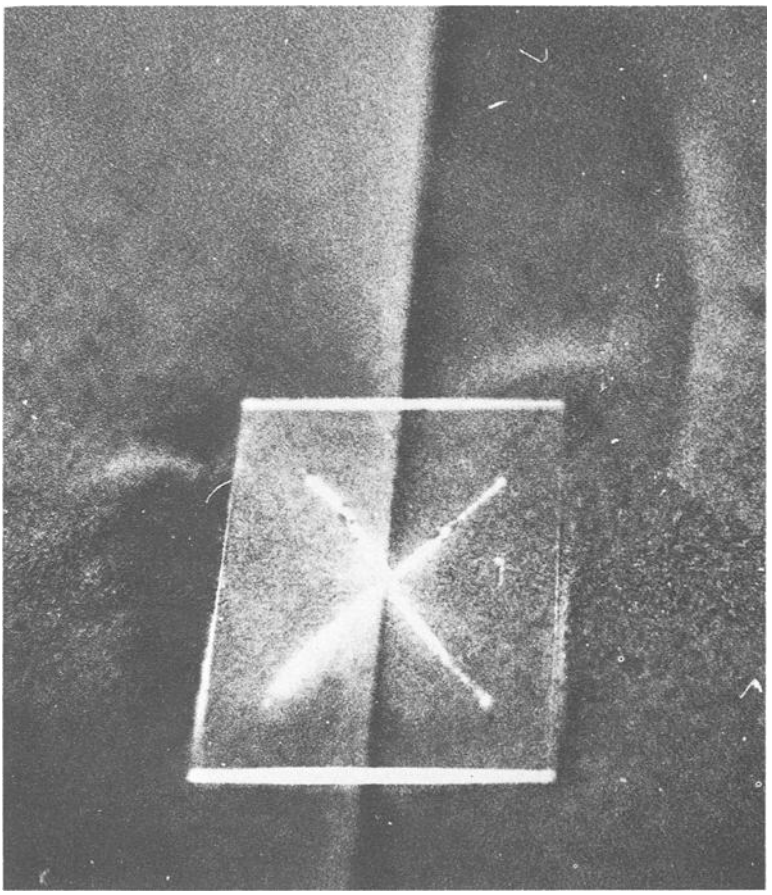

FIG. 2—*Tri-X Panchromatic film, f/1.4, 60 s. Diluted bloodstained "x" on 50 times 75 mm glass slide on brown paper towel on white surface in semi-darkened room.*

was stained with diluted blood. The photographs of these samples when sprayed with luminol reagent accurately reproduced the luminescence perceptible to the eye. Very little that could be visually observed was not recorded on film. Exposure times did not need to be controlled except in the case of extremely bright subjects, where over exposure can occur such as in Fig. 4. When over exposure is not a problem the shutter may be opened, the area sprayed with luminol reagent, and the shutter closed when the luminescence has decayed. As a general rule no exposure need be more than 20 s, even at f/2.8; however, the largest aperture available should be used due to the loss of reciprocity of the film when exposure times exceed 0.5 s. In other words, at these long exposure times the product of the time and the light intensity no longer yields a constant film darkening.

Careful optimization of printing conditions can greatly increase the definition of the luminescent image. A high contrast printing paper such as Agfa Brovvia #6 (F) is especially valuable in improving the quality of the prints. Very faint darkening on the negative, almost imperceptible to the eye, can be printed with this paper.

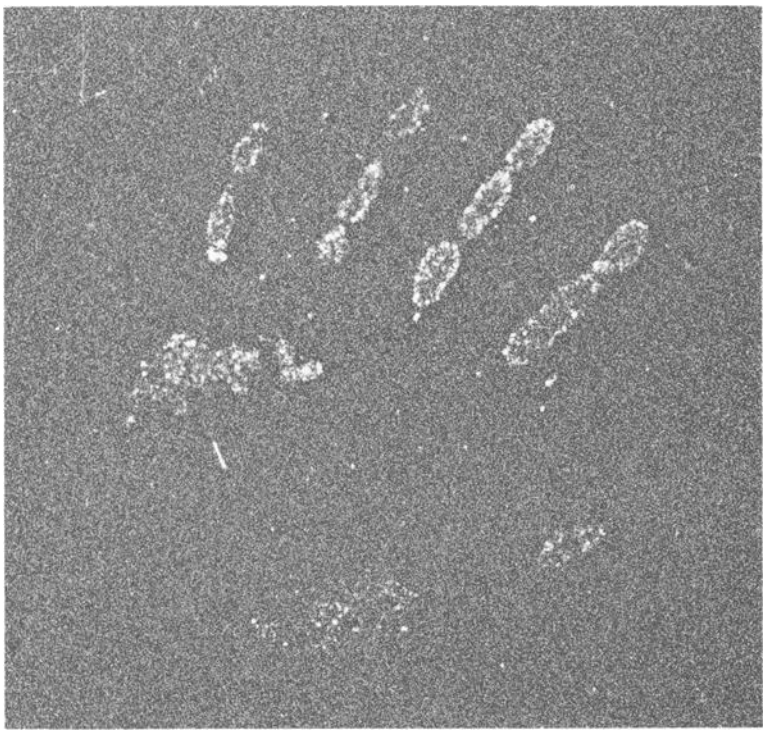

FIG. 3—*Kodak 2475 High Speed Recording film f/2.8, full course of luminescence. Handprint with diluted blood on glass plate. Darkroom conditions.*

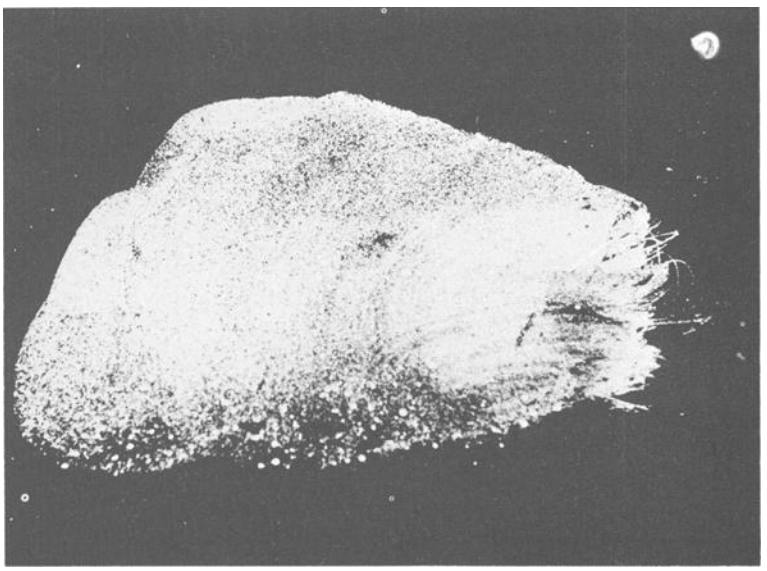

FIG. 4—*Kodak Tri-X Panchromatic film, f/2.8, 30 s. Linoleum flooring with "washed" bloodstain.*

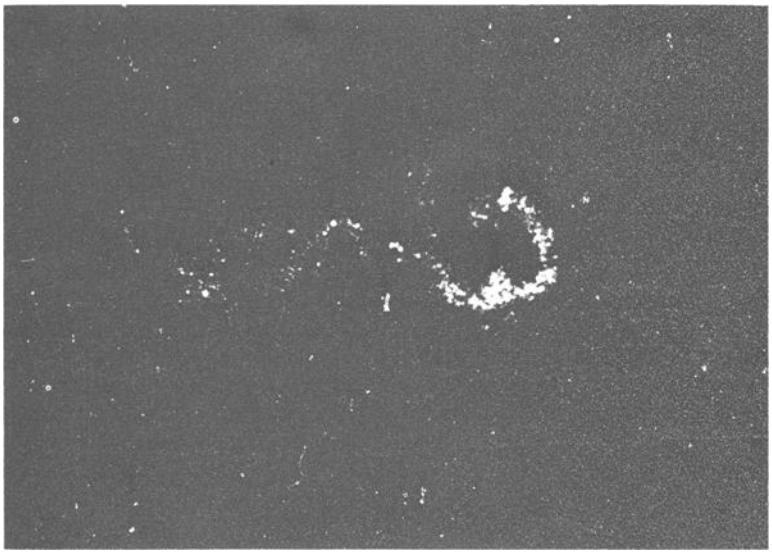

FIG. 5—*Kodak Tri-X Panchromatic film, f/2.8, 30 s. Brick with markings in diluted blood.*

The advantages of employing instant self-developing film are obvious. The photographic parameters may be optimized and the investigator knows if he has useful pictures before leaving the site. Polaroid offers this option in their Type 410 Trace Recording film (ASA 10,000). Photographs with this film at f/4.5 are approximately equivalent to those obtained with 35 mm Kodak Tri-X at f/2.8 (see Fig. 6). The f/4.5 aperture is the largest

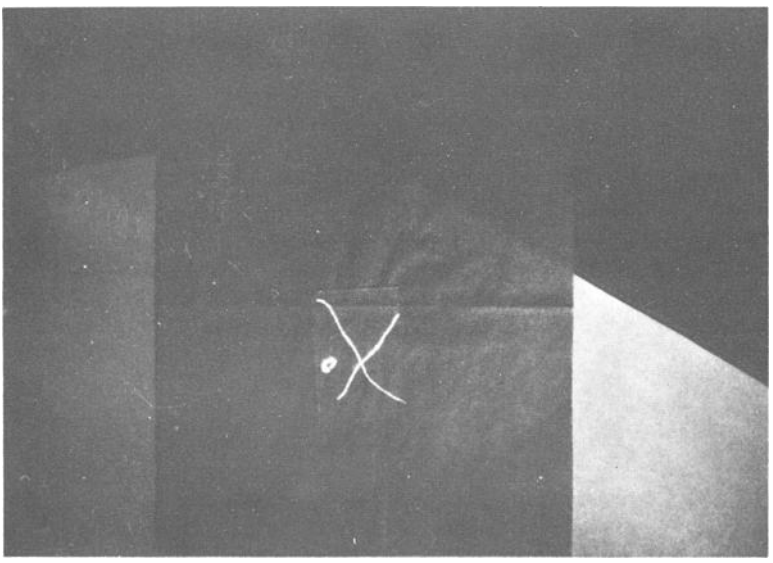

FIG. 6—*Polaroid Type 410, Trace Recording film, f/4.5, 20 s. Blood stained "x" and "o" on 50 times 75 mm glass slide on brown paper towel on white surfaces. Semi-dark room.*

aperture available for the Polaroid camera. There are some limitations to the Polaroid film which do not apply to the 35 mm films. First of all there is no permanent negative; thus any enlargements or additional prints must be made from the print with the attendant loss of definition. Secondly, the final print is determined by the development conditions, that is time and temperature. One cannot, for example, overexpose a print to bring out a weak image as can be done with the 35 mm films.

A Corning glass filter (CS 5-57) polished to 2.5 mm thickness may be used to improve the photography of the luminol reaction in the presence of ambient light. When daylight is the source of ambient light, the exposure time may be doubled without increasing the background, and no detectable loss of the luminol luminescence intensity on the photograph is observed (from the manufacturer's specifications of this filter, a 15 percent loss of intensity for the luminol chemiluminescence would be predicted). Fluorescent lights and mercury vapor lights will probably give less favorable results since several of the more intense mercury atomic emission lines are in the spectral region transmitted by the filter. A red safelight such as that used in a darkroom might be used in conjunction with this filter in order to facilitate the manueverings of the photographer in the dark.

Summary

The performance of several high speed black and white films for the photography of the luminol chemiluminescence test for bloodstains has been evaluated. The application of this technique for providing a permanent record of the location and contours of bloodstains for the forensic investigator is proposed. Several methods of improving the luminescence intensity were investigated.

Acknowledgments

Financial support of this work by the Law Enforcement Assistant Administration and the State of North Carolina is gratefully acknowledged. We are indebted to the members of the North Carolina State Bureau of Investigation for many helpful discussions.

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Research Triangle Institute
P.O. Box 12194
Research Triangle Park, N.C. 27709