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Effects of Luminol on the Subsequent Analysis of Bloodstains

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ABSTRACT: The effects of luminol upon additional presumptive chemical tests, subsequent confirmatory blood tests, species determination by immunoelectrophoresis, ABO typing by absorption elution, and genetic marker analysis by multienzyme system electrophoresis were examined. Results indicate that luminol does not affect additional presumptive chemical tests, confirmatory tests, species determination, or ABO typing, but does affect certain genetic marker systems.

KEYWORDS: forensic science luminol, electrophoresis, genetic typing, blood, bloodstain analysis

Over the last several years, luminol has experienced a rebirth in popularity as a presumptive test for blood [*1–5*]. It has an added distinct advantage in that it may disclose nonapparent bloodstains and patterns of bloodstain distribution. Luminol has generally been used when apparent bloodstains are not found and, generally, as a last resort in the examination of a crime scene. Hence, if blood is found, there usually is not a great deal of it. Frequently, the distribution of bloodstains, for example, wipe marks demonstrating cleaning attempts, is of greater importance than the actual typing of the bloodstains. Obvious bloodstains should never be contaminated with any reagent, including luminol. However, questions have arisen as to the effects of luminol upon bloodstains that are inadvertently sprayed, or upon small amounts of blood discovered with luminol. What are the effects of luminol upon subsequent testing procedures?

Most studies with luminol have indicated that the reagent does not interfere with subsequent confirmatory tests [*6–9*]. However, the studies of luminol's effects on species determination, ABO typing and genetic marker analysis are not as clear. Srch [*10*] reported that luminol interferes with the Takayama confirmatory test, the Lattes test, and the absorption inhibition test. Lee et al. [*11*] reported that luminol affected species testing of bloodstains along with ABO and genetic marker analysis.[2] Recently, Grispino [*9*] reported that luminol had no effect on confirmatory tests by Takayama or species testing by Ouchterlony, but had noticeable effects on ABO typing by absorption elution and on genetic marker analyses.

This study examines the effects of two preparations of luminol upon additional pre-

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sumptive tests for blood, subsequent confirmatory tests, species determination by immunoelectrophoresis, ABO by absorption elution, and genetic marker analysis by multienzyme electrophoresis.

Materials and Methods

Two preparations of luminol were utilized in this study which are designated Luminol I and Luminol II. They were prepared in the following manner:

Luminol I (Grodsky et al. [*12*]):
- 3.5 g sodium perborate
- 0.5 g luminol
- 25 g sodium carbonate
- 500 mL distilled water

Luminol II (Weber [*13*]):
- 10 mL 0.4*N* sodium hydroxide
- 10 mL 0.176*M* hydrogen peroxide
- 10 mL 0.354 g luminol in 62.5 mL of 0.4*N* sodium hydroxide to a final volume of 500 mL (0.004*M*)
- 70 mL distilled water

In this study, the Luminol I solution was prepared fresh prior to use for all experimental protocols. To facilitate mixing, the sodium perborate was dissolved in solution prior to the addition of sodium carbonate and luminol.

The three solutions that make up the Luminol II solution were prepared, refrigerated, and mixed immediately prior to use.

Blood samples used in this study were obtained either from volunteers or case blood standards. Luminol preparations were mixed fresh and sprayed on dried bloodstains with a plastic aerosol bottle in such a manner as to duplicate what is performed at a crime scene. The volume of spray added to each sample was approximately 300 to 500 μL. Spraying was performed in the dark, luminescence was verified, and the moist bloodstains were allowed to dry completely before further analysis. Drying was usually complete within 30 min at room temperature. Samples were stored in the freezer if subsequent analysis was not performed immediately.

Presumptive Blood Tests

Tests were performed to determine luminol's effects on additional presumptive blood methods. Phenolphthalein [*14*], *ortho*-tolidine [*14*], and tetramethylbenzidine [*15*], were used. The inhibition of positive reactions (false negative reactions) by luminol was tested by spraying bloodstains with luminol and then testing the sprayed stains with the reagents and hydrogen peroxide. Whole-blood samples were obtained either from volunteers (five samples) or case samples (five samples) and dried onto either No. 8 thread (SERI) or cotton cloth (SERI). Samples were stored in the freezer if subsequent analysis was not performed immediately. The blood stains were sprayed with the luminol reagents, and the stains were swabbed with cotton-tipped swabs and tested with the presumptive chemical reagents. The swabs were examined for immediate color changes.

The inducement of false positives by luminol was tested by moistening cotton-tipped swabs with the luminol reagents and adding the presumptive chemical reagent, followed by 3% hydrogen peroxide. Again, the swabs were examined for immediate color changes.

Confirmatory Tests

Whole-blood samples were obtained either from volunteers (five samples) or case samples (five samples) and dried onto either No. 8 thread (SERI) or cotton cloth (SERI). The Luminol I and II solutions were applied to the bloodstains and allowed to dry. Small portions of these bloodstains were placed on clean glass slides, coverslips were placed over the samples, and a small drop of Takayama reagent (Ref *16*, Solution 2) was added. The slides were warmed gently and examined microscopically for crystals.

To test for false positives, luminol solutions were sprayed on clean glass slides, which were allowed to dry, and Takayama reagent was added to the slides, as described above. The slides were warmed gently and examined microscopically for crystals.

Species Identification

Three 5-μL samples of whole blood were obtained from volunteers by finger prick, placed onto squares of Whatman No. 3 filter paper, and allowed to dry. The test samples were sprayed with the luminol reagents as previously described, and the squares were allowed to dry. Control samples were not sprayed. The bloodstains were extracted in 1000 μL (1 mL) of distilled water at 2°C in 1.5-mL plastic centrifuge tubes. The extracts were then diluted 1:4 with distilled water.

Rocket electrophoresis was performed according to the method of Rawlinson and Wraxall [*17*] using a barbital buffer system [*18*]. Workers prepared gels by dissolving and bringing to a boil 0.07 g electroendosmosis (EEO) 0.15 agarose (SERI) in 7.0-mL barbital gel buffer (1% gel). When the liquid gelatin cooled to 55°C, 100 μL of anti-human serum (Cappel) was added to the flask, mixed, and poured onto 2 by 3- in. (5 by 7.6 cm) pieces of Gel-Bond film. Holes were cut 1 cm apart at the origin, 1 cm anodic to the cathode using a pasteur pipette. The extracts (2 μL) were carefully pipetted into the wells. Electrophoresis was performed at 40 V for 16 h at room temperature. Gels were pressed for 30 min under Whatman No. 3 filter paper and dried thoroughly in a 60°C oven. The gels were stained in 0.1% Coomassie blue. Peak heights were measured in millimetres.

ABO Typing

Whole-blood samples were obtained either from volunteers or case samples and dried onto either No. 8 thread (SERI) or cotton cloth (SERI). The bloodstains were sprayed with the luminol reagents as previously described and allowed to dry. Control samples were not sprayed. Threads were mounted onto plastic inhibition plates (SERI) with nail polish. Fifteen microlitres of polyclonal anti-A (Ortho), anti-B (Ortho), and H-lectin (SERI) were added to the threads and the plates were refrigerated overnight at 2°C. The threads were washed and blotted several times with paper towels to remove unbound antisera and eluted for 20 min at 60°C in a 0.3% bovine serum albumin solution (BSA). A 0.3% BSA red blood-cell suspension of indicator cells (Ortho) was added after elution and rotated for two 15-min intervals. Agglutination was read and scored microscopically.

Genetic Marker Analysis

The genetic markers esterase D (ESD), phosphoglucomutase (PGM) and glyoxalase (GLO) were typed according to the methods of Wraxall and Stolorow [*19*] in a single system termed Group I.

The genetic markers erythrocyte acid phosphatase (EAP), adenosine deaminase (ADA), and adenylate kinase (AK) were typed according to the methods of Wraxall et al. [*20*] in a single system termed Group II.

The genetic markers hemoglobin (Hb), peptidase A (PEPA), and carbonic anhydrase

II (CAII) were typed according to the methods of Harmor et al. [*21*] in a single system termed Group IV.

The genetic marker phosphoglucomutase was subtyped (PGMs) based on the method described by SERI [*22*] with a modified running time of 3.2 h at 500 V.

The PGM and PGMs gels were preserved after development on Gel-Bond film [*23*]. The overlays for ADA and AK were preserved by placing them on Gel-Bond film, pressing them under Whatman No. 3 filter paper, and drying them at 60°C.

Results and Discussion

Most reports cite A. J. Schmitz as the first to synthesize luminol in 1902 [*24*]. Luminol caught the attention of the forensic science world after the extensive studies of Specht [*6*]. Specht tested fresh and old bloodstains, as well as sperm, saliva, urine, feces, and other body fluids, which were all negative. It is of interest that he tested metals (copper, brass, lead, and zinc) which typically react with luminol, and obtained negative results. Specht also demonstrated the ability to photograph the positive luminol reaction and recommended that it be used for medicolegal examinations.

McGrath [*8*] had favorable comments regarding luminol's apparent specificity but cautioned that it should not be taken as a specific test for blood. Recently, luminol's use in the detection of nonapparent bloodstains at crime scenes has brought it more to the attention of the forensic community [*3–5*].

Luminol's preparation has changed over the years, but it essentially is based on the premise that hemin in blood acts as a catalyst, triggering the oxidation of luminol in an alkaline solution [*7,25,26*].

Presumptive Blood Tests

Both the Luminol I and Luminol II solutions were found to have no noticeable effect on the additional presumptive chemical tests for blood. The luminol solutions did not affect the rate or the intensity of the chemical reactions. No false positives or false negatives were obtained with either of the reagents. Apparently the addition of luminol to bloodstains does not inhibit further oxidation by hemin of reduced-color indicators, that is, *ortho*-tolidine, tetramethylbenzidine, and phenolphthalein. This is consistent with the findings that bloodstains can be made luminescent many times by the reapplication of luminol [*6*].

Confirmatory Tests

Neither the Luminol I nor the Luminol II solution was found to have any noticeable effect on the Takayama test. Hemochromagen crystals were obtained to the same degree and as rapidly as in control samples with both luminol preparations. No crystal formation was obtained with the luminol preparations in the absence of blood.

Species Identification

The results of an experiment designed to test luminol's effects on the species identification of bloodstains are summarized in Table 1. The peak heights of the rockets were measured in millimetres from the center of each well to the top of each peak (Fig. 1). The peak heights represent the total amount of blood-specific antigen present in each sample. Comparison of peak heights of control samples with luminol-sprayed samples revealed that the differences observed were not significant at the 0.05 level of significance based on the chi-square test. The differences observed were most likely due to variations

TABLE 1—*The effects of luminol upon species determination of bloodstains by immunoelectrophoresis.*[a,b]

Luminol I			Luminol II		
Control	Sprayed	X	Control	Sprayed	X
10	9	0.11	13	15	0.27
11	8	1.13	20	15	1.67
12	9	1.00	18	13	1.92
25	21	0.76	21	14	3.50
17	19	0.21			
17	18	0.06	$v = 4$	$X = 7.36$	
19	17	0.24			
20	24	1.50			
20	17	0.54			
27	32	0.78			
$v = 10$	$X = 6.31$				

[a]The distances are in millimetres of Laurell rockets with anti-human antigen.
[b]The differences are *NOT* significant at the 0.05 level of significance.

in sample application. Apparently neither luminol solution reduced the total amount of protein available for species testing. This result is consistent with luminol reacting with hemoglobin and not having any effect on other serum proteins present in bloodstains.

ABO Typing

The results of luminol's effects on the ABO typing of bloodstains by absorption elution are summarized in Table 2. Blind testing was performed on all the samples: a number

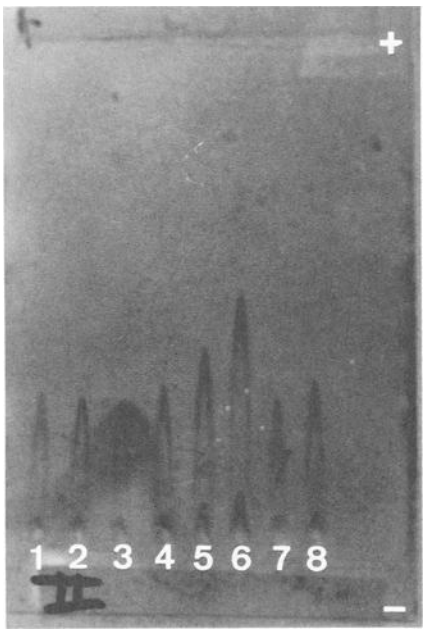

FIG. 1—*Photograph illustrating generation of rockets after immunoelectrophoresis in anti-human serum. Lanes 1, 3, 5, and 7 are control samples. Lanes 2, 4, 6, and 8 are the same samples, which have been sprayed with Luminol I.*

TABLE 2—*The effects of luminol upon the ABO typing of bloodstains.*

Sample	Control	I	II	Sample	Control	I	II
K1	B	B	B	31061-1a	B	B	B
K2	O	O	O	31061-B4	O	O	O
K3	A	A	A	31061-A1	B	B	B
K4	A	A	A	31070-A2	O	O	O
K5	B	B	NT[a]	31399-3	O	O	O
K6	O	O	O	89-0-1586	O	O	NT
PSPC89	O	O	NT	PSP-B-88	B	B	B
PSPD89	B	B	B	32972-1	A	A	A
31061-A3	A	A	A	31985-1	A	NT	A
32668-1	A	A	A	PSPC-88	O	NT	O
32871-C	O	O	O	31196-1	O	O	O
33074-6	A	A	A	31070-A1	A	A	A

[a]NT = not tested.

was given to each sample and the actual bloodtype of each specimen was not known at the time of microscopic examination and grouping. No errors were made in grouping, nor were any noticeable differences observed in the rate of agglutination or degree of agglutination between the control stains and sprayed samples.

Grispino [9] noted a loss in the agglutination intensity, specifically for the A antigen in Group A bloodstains and the B antigen in Group AB bloodstains, as determined by absorption elution using two different preparations of luminol.[3] The detection of the H antigen in Group O bloodstains was not affected by either luminol preparation.

No selective antigen loss was detected in the current study. This apparent contradiction in results may be due, in part, to differences in the methodology, bloodstain size, amount of luminol added, the luminol preparations, and additional differences in the experimental protocol. The results of the current study lead to the conclusion that luminol's chemical reaction with the heme group apparently does not affect the availability of antigen binding sites on the red blood cell membrane.

Genetic Marker Analysis

Both luminol preparations affected certain genetic markers, most notably the Group IV system, but generally the Luminol II preparation had the least deleterious effect.

The Group I system consists of the markers esterase D (ESD), phosphoglucomutase (PGM), and glyoxalase (GLO). Both luminol preparations caused a slight decrease in the band intensity of ESD, but no band shifts. The sprayed bloodstains were typable and consistent with the controls. The PGM banding patterns were occasionally shifted anodically with the luminol reagent, and, generally, the band intensity decreased by approximately one half with both preparations (Fig. 2). The GLO banding patterns showed an occasional loss of band intensity with both luminol preparations, but phenotyping of stains was always possible (Fig. 3).

The Group II system consists of the markers erythrocyte acid phosphatase (EAP), adenosine deaminase (ADA), and adenylate kinase (AK). Most phenotypes in the EAP system were clearly recognizable after spraying with both luminol preparations. A problem arose with the degradation of the intense anodic band of type B, causing it to appear

[3]The Luminol solutions used in Grispino's study [9] were the following: Luminol mixture 1: Part 1 = 0.1 g of luminol and 5 g of sodium carbonate in 50 mL of distilled water; Part 2 = 0.7 g of sodium perborate in 50 mL of 95% ethanol. Luminol mixture 2: Part 1 = 0.1 g of luminol and 5 g of sodium carbonate in 90 mL of distilled water; Part 2 = 10 mL of 3% hydrogen peroxide.

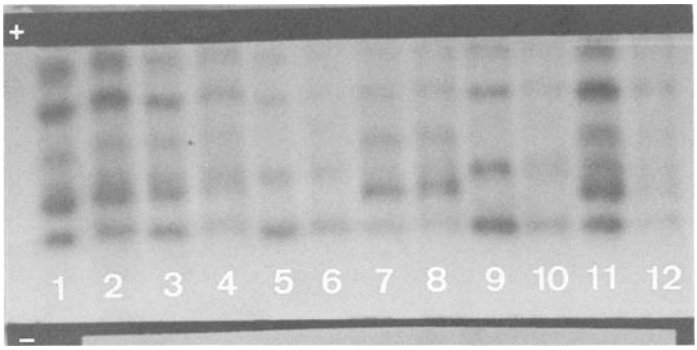

FIG. 2—*Photograph of a Group 1 PGM overlay showing the activity of control and sprayed bloodstains. Lanes 1, 3, 5, 7, 9, and 11 are control stains. Lanes 2, 4, 6, 8, 10, and 12 have been sprayed with Luminol II. Note the loss in activity of some of the sprayed samples.*

as a CB. This degradation and conversion occasionally occurred and has been reported before as a result of thermal degradation of blood samples [*27*]. Caution should be exercised in phenotyping EAP patterns that have been sprayed with luminol. A slight decrease in band intensity occurred with the ADA and AK markers, but phenotyping was possible and no band shifts were observed.

The PGM marker can be subtyped using a conventional electrophoresis system [*22*], and luminol had only slight effects on the banding patterns. A decrease in band intensity of up to one half was occasionally seen with Luminol I but no band shifts were observed.

The most deleterious effects of luminol were on the Group IV system. The migration pattern of hemoglobin was severely distorted by both preparations, and phenotyping was not possible. Luminol I destroyed the activity of PEPA and Luminol II decreased the intensity of the PEPA bands to the point that they were not typable. The activity of CAII was also diminished by both luminol preparations to the point that phenotyping was not possible.

Grispino noted a decrease in band intensity of the PGM subtype marker after spraying with luminol [*9*]. Grispino also reported complete loss of activity with the genetic markers ESD, GLO, PEPA, ADA, and AK. Interestingly, the serum proteins Hp and Gc were not as adversely affected, and all luminol-treated samples were readable.

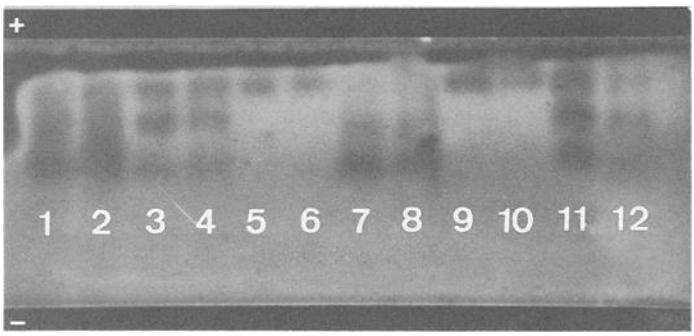

FIG. 3—*Photograph of a glyoxalase overlay showing the activity of control and sprayed bloodstains. Lanes 1, 3, 5, 7, 9, and 11 are control stains. Lanes 2, 4, 6, 8, 10, and 12 have been sprayed with Luminol II. Samples 9 and 11 show a slight loss in activity (Lanes 10 and 12) after luminol application.*

The apparent differences in results between the current study and Grispino's regarding genetic markers may again be due, in part, to differences in the bloodstain size, amount of luminol added, the luminol preparations, and differences in the experimental protocol. It should be pointed out that the electrophoretic procedures used by Grispino were entirely different from those used in the current study.

Conclusions

Both the luminol preparations of Grodsky et al. [*12*], here referred to as Luminol I, and of Weber [*13*], here referred to as Luminol II, showed no deleterious effects on additional presumptive chemical tests, subsequent confirmatory tests, species identification by immunoelectrophoresis, and ABO typing by absorption elution. Depending on the amount of blood detected by luminol and the manner in which it is collected, one may be able to determine the species origin and the ABO grouping of a sprayed bloodstain. The limiting factor will be the sample size.

Both luminol preparations did affect genetic marker analysis to different degrees. A general decrease in band intensity was seen with both preparations but was most noticeable with the Luminol I preparation. It should be noted that this formula is the more concentrated of the two preparations. Even with the decrease in band intensity, phenotyping was still possible with most of the genetic markers. Band shifting was not a significant problem. The decrease in band intensity, however, was most problematic with the EAP marker, and typing in this system should be performed cautiously.

The genetic markers in the Group IV system of electrophoresis were severely affected by both luminol preparations to the point that phenotyping was not possible.

It should be pointed out that no spurious bands were found in either the current study or the one by Grispino after the application of luminol. The luminol reagents, at worst, will render the genetic marker inconclusive, with the possible exception of EAP.

One should keep in mind that luminol is generally used as an investigative tool in cases in which bloodstains are not readily apparent. In such cases, there is probably not a sufficient amount of blood for much more than confirmatory tests and species identification, regardless of the use of luminol. Should luminol be used and lead one to pooled blood, spraying of these stains is not advised. However, should large bloodstains become inadvertently sprayed with luminol, they should be allowed to dry and then collected, since it may be possible to generate useful serological information from these stains.

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References

[*1*] Lytle, L. T. and Hedgecock, D. G., "Chemiluminescence in the Visualization of Forensic Bloodstains," *Journal of Forensic Sciences*, Vol. 23, No. 1, Jan. 1978, pp. 550–555.

[*2*] Gimeno, F. E. and Rini, G. A., "Fill Flash Photo Luminescence to Photograph Luminol Blood Stain Patterns," *Journal of Forensic Identification*, Vol. 39, No. 3, May/June 1989, pp. 149–156.

[*3*] Caporusso, M. K., "Use of Luminol—A Case Study," presented at the poster session, 18th Annual Midwestern Association of Forensic Scientists Meeting, Fairview Heights, IL, 6 Oct. 1989.

[*4*] Laux, D. L., "Detection of Nonapparent Bloodstains with Luminol—Case Studies," presented at the 42nd Annual Meeting of the American Academy of Forensic Sciences, Cincinnati, Ohio, 19–24 Feb. 1990.

[5] Beheim, C. W. and Wolfe, J. R., "The Detection and Comparison of Luminol Enhanced Latent Footwear Impressions," presented at the poster session, 43rd Annual Meeting of the American Academy of Forensic Sciences, Anaheim, CA, 18–23 Feb. 1991.
[6] Specht, W., "The Chemiluminescence of Hemin: An Aid for Finding and Recognizing Blood Stains Important for Forensic Purposes," *Angewandte Chemie*, Vol. 50, 1937, pp. 155–157.
[7] Proescher, F. and Moody, A. M., "Detection of Blood by Means of Chemiluminescence," *Journal of Laboratory Clinical Medicine*, Vol. 24, 1939, pp. 1183–1189.
[8] McGrath, J., "The Chemical Luminescence Test for Blood," *British Medical Journal*, Vol. 2, 1942, pp. 156–157.
[9] Grispino, R. R. J., "The Effects of Luminol on the Serological Analysis of Dried Bloodstains," *Crime Laboratory Digest*, Vol. 17, No. 1, Jan. 1990, pp. 13–23.
[10] Srch, M., "Proof of Blood in Stains After the Application of Luminol Test," *Soudni Lekarstvi*, Vol. 16, 1971, pp. 21–24.
[11] Lee, H. C., Gaensslen, R. E., Pagliaro, E. M., and Guman, M. B., "The Effects of Reagents for Presumptive Blood and Body Fluid Testing, Latent Fingerprint Enhancement and Histologic Stains on Subsequent Serological Tests," presented at the 38th Annual Meeting of the American Academy of Forensic Sciences, New Orleans, LA, 10–15 Feb. 1986.
[12] Grodsky, M., Wright, K., and Kirk, P. L., "Simplified Preliminary Blood Testing. An Improved Technique and Comparative Study of Methods," *Journal of The American Institute of Criminal Law and Criminology*, Vol. 42, 1951, pp. 95–104.
[13] Weber, K., "Die Anwendung der Chemiluminescenz des Luminols in der Gerichtlichen Medizin und Toxicologie. I. Der Nachweis von Blutspuren," *Deutsche Zeitschrift für die Gesamte Gerichtliche Medizin*, Vol. 57, 1966, pp. 410–423.
[14] Saferstein, R., Ed., *Forensic Science Handbook*, Prentice-Hall, Inc., Englewood Cliffs, NJ, 1982.
[15] Holland, V. R., Saunders, B. C., Rose, F. L., and Walpole, A. L., "A Safer Substitute for Benzidine in the Detection of Blood," *Tetrahedron*, Vol. 30, 1974, pp. 3299–3302.
[16] Gaensslen, R. E., *Sourcebook in Forensic Serology, Immunology, and Biochemistry*, Superintendent of Documents, U.S. Government Printing Office, Washington, DC, 1983.
[17] Rawlinson, L. and Wraxall, B., "Semen Quantification Utilizing P30 Antigen," presented at the Interamerican Congress of Forensic Sciences, Sacramento, CA, 2 Nov. 1982.
[18] Culliford, B. J., "A New Method for Precipitin Reactions on Forensic Blood, Semen, and Saliva Stains," *Nature*, Vol. 201, 1964, pp. 1092–1094.
[19] Wraxall, B. G. D. and Stolorow, M. D., "The Simultaneous Separation of the Enzymes Glyoxalase I, Esterase D, and Phophoglucomutase," *Journal of Forensic Sciences*, Vol. 31, No. 4, Oct. 1986, pp. 1439–1449.
[20] Wraxall, B., Bordeaux, J., Harmor, G., and Walsh, J., "Final Report—Bloodstain Analysis System," Report No. 025-73, Law Enforcement Assistance Administration, U.S. Department of Justice, Washington, DC, July 1978.
[21] Harmor, G., Wraxall, B., and Blake, E., "Multisystem Approach to Red Cell Black Population Markers—Group IV," Southern Forensic Association, Savannah, GA, 1982.
[22] Wraxall, B. and Provost, J., "PGM Subtyping by Conventional Electrophoresis," presented at the Combined SAFS, MWAFS, MAFS, and NEAFS Meeting, Louisville, KY, 9 May 1980.
[23] Laux, D. L., "Preservation of Gels After Electrophoresis," *Bulletin of the Midwestern Association of Forensic Scientists*, Vol. 2, No. 1, March 1983.
[24] Schmitz, A., "Uber das Hydrazid der Trimesinsaure und der Hemimellitsaure," dissertation, Heidelberg, published by J. Horning, 1902; cited by Gaensslen, R. E., *Sourcebook in Forensic Serology, Immunology, and Biochemistry*, U.S. Government Printing Office, Washington, DC, 1983.
[25] Erdey, L., Pickering, W. F., and Wilson, C. L., "Mixed Chemiluminescent Indicators," *Talanta*, Vol. 9, 1962, pp. 371–375.
[26] Thornton, J. I. and Maloney, R. S., "The Chemistry of the Luminol Reaction—Where to from Here?" *California Association of Criminalists Newsletter*, Sept. 1985, pp. 9–16.
[27] Wraxall, B. G. D. and Emes, E. G., "Erythrocyte Acid Phosphatase in Bloodstains," *Journal of the Forensic Science Society*, Vol. 16, 1976, pp. 127–132.

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