

# Direct Sensitivity Comparison of the Fluorescein and Luminol Bloodstain Enhancement Techniques

*Rob Cheeseman*

*Forensic Consultant  
Bonita, CA*

**Abstract:** The scope of this study is to conduct a direct sensitivity comparison between the refined Fluorescein and Luminol bloodstain enhancement techniques. This comparison will be subjective in nature and will examine bloodstain sensitivity of each bloodstain enhancement technique relative to each other on various substrates (porous and nonporous). The Fluorescein technique was obviously more sensitive, and the results were tabulated as a *sensitivity multiple*. The results collected were indicative of the Fluorescein technique being at least twice (2x) as sensitive, and perhaps as great as five times (5x) as sensitive as Luminol.

## **Introduction**

The goal of this study is to investigate a direct sensitivity comparison between the fluorescein and luminol bloodstain enhancement techniques on various substrates. A discussion of similarities and differences resulting between these two techniques will be addressed and documented, as will the possibility of augmenting the fluorescein technique with other confirmational tests.

Previous literature has listed many different bloodstain enhancement reagents; however, as yet, the author is not aware of any studies

which demonstrate a *direct* sensitivity comparison. Indeed this study will only demonstrate the sensitivity of fluorescein relative to lumimol. One article in particular compared numerous reagents/techniques relating their respective sensitivities. However, some of the results compared various studies performed at various times by numerous investigators. Accordingly, it was remarked that the theoretical possibilities cited did not reflect the practical results they found of the reagents which they studied [1].

Since the introduction of the modified fluorescein technique and its subsequent refinements, the author has performed and documented numerous direct comparisons as previously mentioned, and has found that the theoretical and practical results were not reflective. Initially, personal technique was considered as a factor which might account account for the luminol results being less sensitive; however, performance by other well-experienced senior personnel yielded similar results.

Both fluorescein and luminol techniques subscribe to the same chemical principle. Both are *peroxidase-reductions* reactions targeting hemassociated molecules (enzymes and iron) found in the red blood cells (RBCs), as are many other presumptive blood reagents (i.e., phenolphthalein, benzidine, leucomalachite green, etc.). All use a relatively colorless chemical reagent in its reduced state sprayed onto a targeted or suspected area which is chemically altered in the presence of hemoglobin (peroxidase and iron) to its colored or oxidized state. In the circumstance of luminol, its oxidized state is expressed by a chemoluminescent burst, and in the case of fluorescein, its reduced state is *fluorescein* which is oxidized to *fluorescein* [2].

This expression is colorimetric, and under certain conditions (excited by an ALS @ approximately 450nm) has the ability to fluoresce [3]. It is probably this fluorescent capacity which allows the fluorescein technique to yield greater sensitivity, due to increased ambient light, and most certainly the documental advantages.

## Procedure

The materials and methods for this study are standardized and listed in the literature and references; hence, they will not be cited here [4, 5]. Most of the data were gleamed during the fluorescein study [4]. The duration of the study contained five separate experimental sessions of

comparisons which were conducted on various substrates. The preparation of the fluorescein and luminol reagents was performed by one investigator but, to ensure the quality assurance of the luminol technique, the last experimental session was prepared by senior and well-seasoned investigators to ensure valid results.

In the first and second experimental sessions, the fluorescein technique did not contain the commercial thickener. However, the remaining experimental sessions did, and the results are listed.

Various porous and nonporous substrates were utilized for this comparison (blotter board, smooth and textured masonite, and glass) to simulate differing potential crime scene substrate surfaces. The blood dilutions of positive results ranged as high as 1:12,000; however, the issue of sensitivity and the normal ranges of hematocrit versus absolute values will be addressed in the discussion section. The reactions of the bloodstain enhancement techniques were subjectively judged by two or more investigators, and the results were rated from negative / zero (0) to four (4) plus / positive with the weak reactive results designated by a +/- symbol.

After the initial performance parameters of the fluorescein technique were better understood, the dilution intervals were altered throughout the latter sessions to improve the precision of the results, and ultimately to improve accuracy. Only the sensitivity differential multiple is listed below and this issue will be addressed in the discussion section.

## Results

Since the intent and scope of this study is only to compare the relative sensitivities between fluorescein and luminol, the following is a tabulation of the sensitivity differential between the two techniques. Consistently, the fluorescein technique yielded greater sensitivity over luminol (as listed below) with increased sensitivity following the addition of the commercial thickener to the fluorescein technique.

**Table 1**

Experimental Session/Run	Type Substrate	Sensitivity Multiple Increase
1/1	Blotter board (porous)	2x
1/2	"	2x
1/3	"	2x
2/1	"	2x (+/-)
3/1	Masonite (nonporous)	4x
3/2	"	2x
3/3	"	2x
3/4	"	2x
4/1	Blotter board (porous)	5x*
4/1	"	5x*
5/1	Glass (nonporous)	3x*
5/2	"	3x*

\* Addition of commercial thickener to the protocol

Some of the results in the early experimental sessions may have been influenced by the dilution intervals which were changed in the later experimental sessions to gain greater precision. The improved sensitivity of the fluorescein results were consistent. This consolidates the belief that the fluorescein technique is at least twice (2x) as sensitive as luminol and as great as 5x (averaging 4x) more sensitive than luminol in accordance with these results.

### Discussion and conclusions

The original intent and purpose of this data were to demonstrate that the fluorescein technique sensitivity was comparable to luminol but, early on it was noted that the fluorescein technique yielded an increased sensitivity over luminol. The consistency of the results collected during five experimental sessions and twelve runs were indicative that the fluorescein technique is more sensitive even through some literature listed luminol relatively more sensitive [1].

All of the data for this study were collected during the development and refinement phase of the fluorescein technique; hence, the scope of the subjective observations was only intended to express a comparability to luminol (an established sensitive technique) [4]. A more desirable approach would have been to utilize an instrumentational objective approach; however, more recent literature lends itself well to this point and has shown that luminol, when augmented with fluorescein, yields a four fold (4x) increase in sensitivity [6].

In the final analysis, whether or not the reaction of a given technique is discovered via objective instrumentation or subjective observation, it will be ultimately detected by the subjective observation of the investigator's eye. And for these purposes, the scope of the methodology chosen is adequate for this intent in this study.

To address the issue of bloodstain detection sensitivity and statistically sound numbers, the target of the peroxidase-reduction principle are hemassociated molecules specifically proteins (enzymes) and iron ions ( $Fe^{2+}/Fe^{3+}$ ) which are found in red blood cells (RBC's). A normal variance of over 25% for the hemoglobin levels or hematocrit may exist.

The hematocrit is the ratio of red blood cells (RBC's) per given volume of whole blood expressed as a percentage, and may range within normal healthy subjects to as low as 36% for females, to as high as 50% in males. This range is dependent on sex, age and/or physical health of the subject within a given population. Consequently, this normal but vast range can be statistically problematic. Statically sound numbers are difficult to obtain even in a "normal population", and more so when including subjects which are not within the normal variance in which victims and/or suspects may well be. The worst case scenario is an anemic (low hematocrit) subject contrasting with that of a normal healthy subject. For this study all the blood dilutions were donated by a singularly healthy subject (the author) for comparative purposes. Naturally, blood from a different source may produce different *absolute* values; however, the *relative* values would yield the same results between the two bloodstain enhancement techniques. Consequently, the results listed are cited as a *differential multiple*.

Another observation noted during these experiments was that the fluorescein and luminol bloodstain enhancement techniques can be serially administered. This empirical exercise was successfully demon-

strated during experimental session #4. Subsequently, this author has been called upon to perform an examination in a case in which evidence had already been processed with luminol. Second chances are rare in forensics, and it is not recommended that this become a tactic. If for whatever reason, there are circumstances in which the documentation of one or the other technique was not successful, either fluorescein or luminol can be serially administered and become the other's alternative.

In such an event, the smallest minutia may be lost due to dispersion of a wetted bloodstain; however, large or gross minutia may still be attainable and documentable. Also it has been observed during empirical exercises and actual case work that the fluorescein technique can be administered *after* a target area has been dusted for fingerprints. Processing with *protein stains* (amido black and coomassie blue) to attain fingerprint evidence *after* the fluorescein procedure (via a misting rinse of distilled water to remove the fluorescein/thickener), has been empirically demonstrated.

Further processing is possible in reference to DNA analysis. Some aspects of the fluorescein procedure are favorable to the DNA polymerase chain reaction (PCR) amplification technique [7]. Empirical exercises have been conducted to determine if the fluorescein technique would have any detrimental effects on PCR analysis, and none were observed. Moreover, DNA evidence has been successfully collected via fluorescein detection and amplified in two murder cases to date. However, this is by no means a guarantee that DNA analysis will be successful at every crime scene in which the fluorescein technique is employed.

The ratio of RBC's to WBC's can be higher than 1400:1 in a normal healthy male; hence, there are many more RBC's than WBC's. The PCR and fluorescein techniques targets different components in the blood; hence, these techniques are not analogous, and it is completely possible to have a positive fluorescein result yet have the DNA analysis be unsuccessful. For technical reasons it is quite likely that success in DNA analysis is the exception rather than the rule. However, it can be done and can be very important to the investigation when it is successful. Let it be noted that DNA analysis targets the nuclear material in the white blood cells (WBC's). Both of these techniques are probably inversely proportional to each other but, fundamentally different and not analogous to each other.

A sensitive, reliable and safe bloodstain enhancement procedure is an invaluable asset in the analysis of a crime scene or crime scene reconstruction. By the time this study is published the fluorescein technique reagents will be commercially available in a kit format. The fluorescein technique has demonstrated an average of approximately four fold (4x) increased bloodstain detection sensitivity as compared to luminol.

The fluorescein technique bloodstain pattern retention (even on vertical nonporous surfaces) is now possible due to the utilization of a commercial thickener yielding an improvement in bloodstain pattern interpretation. Documentational techniques have been broadened (to include video and digital photography to augment traditional photography) due to the increased amount of ambient light from the ALS. All of these factors have produced a quantum improvement in bloodstain detection and enhancement as a result of the refined fluorescein methodology, thus expanding from a somewhat mostly investigative procedure to a more evidentiary technique. This has broadened the scope of application to include situations in which previous bloodstain enhancement techniques would not have been thought appropriate and/or effective. Ultimately, it is hoped that these improvements will result in better investigations, producing clearer court presentation and understanding by the trier of fact.

### **Acknowledgments**

The author extends his gratitude to the staff of San Diego Sheriff's Department Regional Crime Laboratory (Randy Robinson and Marty Fink), San Diego Police Department (Gary Avery and staff) and the Escondido Police Department (Detective George Durgin and staff) for their support during this study.

For further information, please contact:

Rob Cheeseman  
Forensic Consultant  
4844 Camino Roberto  
Bonita, CA 91902  
(619) 470-8787  
rcheeseman@juno.com

## References

1. Olsen, Sr., R. D., "Sensitivity Comparison of Blood Enhancement Technique", *Identification News*, 35(8) 1985, p 10.
2. Thornton; Maloney, "The Chemistry of the Luminol Reaction – Where to From Here" Forensic Science Group, Dept. of Biomedical and Environmental Health Science, University of California, Berkeley, CA.
3. Monk, J. W., "Fluorescent Bloodstain Detection - A Replacement for Luminol", Msc project report to CCI, 1991.
4. Cheeseman, R.; DiMeo, L. A., "Fluorescein as a Field-worthy Latent Bloodstain Detection System", *Journal of Forensic Identification*, 45(6), 1995, pp 631-646.
5. Saferstein, R., "*Criminalistics an Introduction for Forensic Science*" fifth edition, chapter 12, p 355.
6. Kalkar, C. D.; Doshi, S. V.; Pendse, M., "Aqualuminescence of alkaline luminol in the presence of fluorescein", *Int J Rad Appl Instrum [A]*, 37(1):41-5 1986.
7. "*Biotechniques*" 21:392-394 (September 1996)