ILLUMINATING LATENT BLOOD

Application methods, fixatives, alternatives and new formulas for luminol

Morgan Cheyne

A thesis submitted in fulfilment of the requirements for the degree of Master of Science
in Forensic Science, The University of Auckland, 2011

ABSTRACT

Luminol is an effective chemical reagent for the detection and enhancement of latent blood and is widely utilised for this purpose throughout the forensic community. It is characterised by a pale blue chemiluminescent light which is emitted upon reaction of luminol with blood.

Many improvements to the luminol formula have been attempted over the years. In this thesis I critically evaluated three new luminol based formulas, one commonly used luminol formula and an alternative to luminol, fluorescein. These reagents were compared and contrasted in terms of sensitivity, longevity of reaction and DNA preservation as well as economic and practical considerations.

Blood pattern distortion and destruction is a major disadvantage to using luminol at crime scnenes. Five spray types and five fixatives/ shear thinning agents were evaluated on their ability to preserve the spatial morphology of bloodstain patterns on non-porous surfaces.

Lumiscene Ultra showed the highest intensity of emitted light for higher concentrations of blood. However, when blood was diluted to lower concentrations, this intensity was comparable to Bluestar Magnum, Lumiscene and Hemascein blood detecting reagents. All of the aforementioned reagents, however, had a greater sensitivity than the Grodsky formula. Hemascein had the longest reaction time with Lumiscene Ultra and Grodsky having the longest reaction times for the luminol based reagents. All of the reagents showed a certain amount of DNA degradation when compared to a water control sample. Hemascein preserved DNA to a greater extent than the rest of the reagents. Lumiscene significantly decreased the success of DNA profiling success.

The ECO spray and spray gun were found to be the best application methods for luminol for the purpose of preserving the morphology of blood patterns. The hand pump sprayer severely affected the preservation of blood patterns.

The combination of a zinc fixative, a shear thinning agent called ABA fix and the ECO spray was found to be best at fixing and preserving the morphology and spatial position of blood patterns.

LOD 5 AU	10	4-4.5	4.5-5	-	-

Table 4.6: Shows the limit of detection for each of the reagents at 10 and 5 intensity units.

From the above table it can be seen that Bluestar Magnum has the shortest reaction length. Lumiscene had a slightly greater LOD than Bluestar Magnum at both 5 and 10 arbitrary units. Grodsky had a longer significantly longer reaction length than Bluestar Magnum and Lumiscene. Lumiscene Ultra, contrary to the images, appeared not to have a limit of detection as the intensity of the reaction at 20 minutes was greater than 10. Hemascein also had an intensity value greater than 10 after 20 minutes with an intensity value considerably greater than that of Lumiscene Ultra.

4.3 DNA and mRNA Analysis

4.3.1 DNA Quantitation

DNA Quantitation was important in this study, first to determine the amount of human DNA present to determine the amount of DNA solution that should be added to the PCR tube for STR amplification. The Identifiler™ PCR amplification kit requires a final input DNA concentration of 0.05-0.125ng/µl. If the input DNA is less or more than this range then STR profiling will be impaired (AmpFLSTR® Identifiler® User's Manual.

Also, DNA quantification is important to determine whether there are inhibitors present which could inhibit the PCR reaction. The Quantifiler™ kit contains an internal PCR control (IPC) sequence which is co-amplified with the sample. The IPC is a synthetic oligonucleotide sequence which produces fluorescence which is detected by the quantification system. The IPC is useful for determining whether the DNA present in the sample is unsuitable for amplification by PCR by predicting the presence of PCR inhibitors. If PCR inhibitors are present the IPC is either not amplified or is amplified with a high Ct value. If there is no human DNA present, or the DNA is degraded, the IPC is still amplified. (M. Barbisin et al Validation of a multiplexed system for quantification of Human DNA and human male DNA and detection of PCR inhibitors in biological samples (2007) Proceedings of the 18th