

**CHEMICAL ENHANCEMENT OF WASHED AGED BLOODSTAIN ON
POROUS MATERIALS USING LEUCOCRYSTAL VIOLET**

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*Specially dedicated to my parents, Hj. Pakri Mohamed bin Mohd Ismail and Hjh
Mahau Mutha binti Abdul Karim*

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ABSTRACT

Blood is an important physical clue material encountered in the cases involving physical violence like murders, assaults, rapes, abortion etc. The examination of bloodstains is of immense value in reconstruction of the scene of crime and linking criminal or the victim with the scene of crime. As criminals now often attempt to clean up the crime scene and it is not known through which conditions the bloodstain has undergone before analysis. The main aim for this study is to investigate the use of leucocrystal violet to develop faint bloodstains from porous materials which will be washed with a commonly available cleaning product. Keeping this aspect in view, bloodstains have been examined after exposure to leucocrystal violet and Luminol (for comparison purpose) in order to assess their effect on the detect ability of serological markers as in blood grouping analysis. This study shows that leucocrystal violet has no destructive effect of samples as well as on elution method for the detection of blood group antigens. However, luminol did show an adverse effect on subsequent blood analysis. Thus, it is concluded that luminol has the most adverse effect on the ABO blood grouping. Based on four cloth matrices that were subjected to chemical enhancements, cotton showed the best chemical enhancement result cumulatively. This is due to chemical composition of cotton that allows chemical reactions to occur. In conclusion, positive identification in case of cloth materials that have been washed with bleaching agent and enhanced with Luminol is less accurate and less reliable to compare with samples treated with Leucocrystal violet in the same condition.

ABSTRAK

Darah merupakan bahan kes yang penting dan sering dijumpai di dalam kes jenayah seperti kes pembunuhan, kes dera, kes rogol, kes pengguguran anak dan lain-lain. Darah yang dikenal pasti di tempat kejadian adalah sangat penting untuk rekonstruksi tempat kejadian dan sekaligus untuk mengaitkan pesalah laku ataupun mangsa ke tempat kejadian. Pesalah laku pada hari ini sering cuba untuk membersihkan tempat kejadian dan keadaan darah tidak ketahu sebelum analisa dilakukan. Kajian ini dilakukan untuk mengkaji kegunaan leucocrystal violet untuk proses penyerlahan kesan darah yang cair daripada kain yang dibersihkan dengan agen pembersih. Dengan ini, darah diperiksa setelah proses penyerlahan dengan menggunakan leucocrystal violet dan luminol dilakukan untuk mengenalpasti kesan bahan kimia ke atas analisis darah. Kajian ini menunjukkan, leucocrystal violet tidak mempunyai kesan yang membinasa ke atas sampel dan analisis darah. Walau bagaimanapun, luminol menunjukkan kesan negatif ke atas analisis darah ABO. Berdasarkan keberkesanan empat jenis kain yang digunakan dalam kajian ini, kain jenis kapas yang menunjukkan kesan penyerlahan yang baik secara menyeluruh. Ini adalah disebabkan kandungan kimia kain tersebut yang mengizinkan reaksi kimia untuk berlaku. Kesimpulannya, penemuan positif di dalam kes yang mengaitkan kain yang telah dibersihkan dengan agen peluntur dan mengalami proses penyerlahan darah menggunakan luminol adalah kurang tepat dan kurang kukuh berbanding dengan sampel yang mengalami proses penyerlahan darah menggunakan leucocrystal violet dalam keadaan yang sama.

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LIST OF ABBREVIATIONS

MSDS	-	Material Safety Data Sheet
LCV	-	Leucocrystal Violet
DNA	-	Deoxyribonucleic Acid
RMP	-	Royal Malaysia Police
DFO	-	1,8, Diazaflouren-9-one
Nm	-	Nanometer
Cm	-	Centimeter
mL	-	Milliliter
rpm	-	Revolution per minute
μL	-	Microliter
g	-	Gram
°C	-	Degree Celcius

CHAPTER 1

INTRODUCTION

1.1 Preamble

A successful crime scene investigation depends upon the collection and analysis of various kinds of evidence. Locard's Exchange Principle dictated that evidence, both physical and biological, is to be found at the scene of a crime because the perpetrator always leaves something behind by having contact with victims and objects at the crime scene (Dwane, 1995). One of the most vital biological evidence is blood. Blood is one of the most common and important physical evidence used by the investigator to link a perpetrator to a crime. Bloodstain evidence is considered as highly valued form of physical evidence commonly found at scenes involving violent crimes.

One method of detecting faint or dilute bloodstains includes spraying the area suspected of containing bloodstains with a chemiluminescent agent (5-amino-2, 3-dihydro-1,4-phthalazine, also known as luminol) and then detecting emitted light where the blood is located. Oxidation of luminol is accompanied by a striking emission of light that can be visually or photographically detected. Oxidation of luminol occurs in the presence of iron and peroxides, both of which are generally present in bloodstains.

Because of this property, luminol has been one of the most commonly used bloodstain detection reagents. Luminol can also be oxidized by environmentally-present iron, copper, cyanides and peroxides, which can result in high background signals when attempting to locate latent bloodstains in some environments.

In addition, luminol has been characterized as a "possible carcinogenic" on material safety data sheets (MSDS) provided by commercial suppliers to provide consumers with safety information. Because of increased concern by users and regulatory agencies, such as the Environmental Protectional Agency, with respect to toxic and/or mutagenic reagents used in the work place, there is a need for an alternative to luminol for detection of latent bloodstains. Enforcement of more stringent safety guidelines may also have limited the use of luminol. Thus, there is a need for a safe, reliable alternative bloodstain enhancement technique.

Leuco-crystal Violet (LCV) is another commonly used latent blood reagent for evidence and crime scenes. Like luminol, the application of LCV to latent bloodstains creates a catalytic reaction with haemoglobin. Unlike luminol, however, the LCV reaction is visible in normal lighting. LCV stain latent blood a dark purple to black color allowing for easy observation and documentation on light colored surfaces.

1.2 Problem Statement

Luminol is used by crime scene investigators to locate traces of blood, even if it has been cleaned or removed. The investigator prepares a solution of luminol and the activator and sprays it throughout the area under investigation. The iron present in any blood in the area catalyzes the chemical reaction that leads to the luminescence revealing the location of the blood. The amount of catalyst necessary for the reaction to occur is very small relative to the amount of luminol, allowing the detection of even trace amounts of blood. The glow lasts for about 30 seconds and is blue in color. Detecting the glow requires a fairly dark room.

However, the uses of luminol have some drawbacks that may limit its use in crime scene investigation. Luminol chemiluminescence can be also triggered by a number of substances such as copper or copper-containing alloys, and certain bleaches; and, as a result, if a crime scene is thoroughly cleaned with a bleach solution or horseradish, residual cleaner will cause the entire crime scene to produce the typical blue glow, effectively camouflaging any organic evidence, such as blood.

Besides that, luminol will also detect small amounts of blood presence in urine, and it can be distorted if animal blood is present in the room that is being tested. Luminol's presence may prevent other tests from being performed on a piece of evidence. However, it has been shown that DNA can be successfully extracted from samples treated with luminol reagent.

On the other hand, leucocrystal violet is known as blood detector. Leucocrystal violet is the completely reduced form of Crystal Violet and is therefore colorless. When LCV and hydrogen peroxide come into contact with the hemoglobin in blood, a catalytic reaction occurs and the solution turns to a purple/violet color. When bloody footwear impressions are visually located or otherwise suspected at a crime scene, LCV application provides a quick and uncomplicated method of visualizing and enhancing those impressions. The application of LCV, particularly to large crime scene areas in most scenarios, has several distinct advantages over Amido Black, DAB and Luminol. It is easy to mix and, with reasonable safety precautions, does not present any significant health hazards. It is easy to apply with pressurized spray devices. It results in almost instant visualization of impressions which in turn enables that information to be incorporated in photographs and crime scene notes and sketches. In most instances, LCV provides sufficient additional visualization to warrant additional examination quality photographs be taken.

The chemical methods developed for the enhancement have associated challenges. It should be noted that any of the chemical methods used must not destroy or prevent subsequent blood group typing and DNA analysis. Caution is therefore recommended when using an enhancement technique on possible bloody

materials to ensure that sufficient biological material is retained by the substrate for possible future blood group typing analysis and DNA submission. Besides that, the chemical techniques applied may be issued based on their sensitivity, stability as well as their ability to preserve the biological details. The most important challenges are to ensure that all these methods are relatively safe for users.

1.3 Hypothesis Statement

The washed bloodstains are enhanced using LCV reagent. The enhanced bloodstains are further subjected for blood analysis to find out the effect of LCV on blood grouping analysis.

1.4 Objectives of the Study

The objectives of this study are:

- i) To investigate the use of LCV to develop latent bloodstains from porous materials which has been washed with a commonly available cleaning product.
- ii) To determine the effects of chemical enhancement methods on blood grouping analysis.
- iii) To compare the effectiveness of currently practiced blood enhancement method, Luminol with LCV reagent.

1.5 Significance of Study

This study will give an idea for the crime scene investigator in handling the possible latent bloodstains obtained from various types of surfaces which often found or recovered in violent crimes. Blood is the most valuable evidence in the crime scene which need to be preserved for further identification. From this study, the best method to enhance bloodstains on washed porous materials could be obtained. The results of this study will benefit the investigative officers of the Royal Malaysia Police (RMP) and would be useful to the forensic scientist at Jabatan Kimia Malaysia or in any private consultation companies.

1.6 Scope of Study

This study involves the projection of latent bloodstains on various porous materials. The research also covers chemical enhancement methods which capable to enhance possible bloodstain pattern on washed porous materials

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