Laten Blood Detection Test Sensitifity of Lizard (*Varanus salvator*) Using Leuco Malachite Green (LMG) with Different Dilution

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Keywords: Forensic, Forensic veterinary, Lizard, Blood Pattern staining, Leuco Malachite Green, LMG

Abstract: This study was undertaken to investigate reaction sensitivity blood detection Leuco Malachite Green (LMG) of *Varanus salvator's* blood. Forensic veterinary may be requested in both criminal and civil cases. Blood tests at crime scene can provide useful information for the investigation process. Indonesia is home to several varanid species. *Varanus salvator* known as water lizard. This animal is an endemic lizard species of Indonesia. Leuco Malachite Green is one of latent blood staining method that commonly used to detect blood pattern on the crime scene or tools. The aim of research is to compare the sensitivity of LMG reaction to each different blood varanus salvator dilution (10^1, 10^2, 10^3, 10^4, 10^5). 35 total samples were used in this study. The staining results show different sensitivity to different dilutions. The higher the dilution rate becomes less visible.

1 INTRODUCTION

Blood tests at crime scene can provide useful information for the investigation process (Idries *et al*, 2011). Blood is the most common and perhaps the most important form of evidence in today's criminal justice world (Tobe *et al,* 2007). An investigator can interpret certain basic patterns at the scene, such as drag marks, smears, or blood trails. It is important to keep in mind that an injured animal may be mobile and may shake his head or body, causing spatter (Merck, 2007). Mature reptile erythrocytes are generally larger than bird erythrocytes or mammals. The reptile erythrocytes are ellipsoid cells with an oval or rounded middle core. The color of the cytoplasm is more pink uniform with the staining of Wright's Stain (Bijanti *et al*, 2010).

Presumptive tests for blood utilize a variety of chemicals to identify the presence of blood through a reaction with the haemoglobin molecule (Spalding, 2006). They are described as presumptive because there are substances other than haemoglobin which may cause a false positive reaction and in forensic settings further testing is required to confirm the result. These are rapid tests that are used to identify whether an unknown substance is likely blood and to identify areas of a crime scene that should be investigated in more detail. The benefit of utilizing these tests is the rapidity of results and the ease of interpretation (Colotelo, 2009). Leuco Malachite Green (LMG) is widely used for presumptive testing in casework at crime scene investigations and in the laboratories. A presumptive test will indicate if a biological substance such as blood is present in a stain found (Andersson, 2017). Hemoglobin makes up the greater share of the solid content of the red blood cell (Dessauer, 1970). Heme-reacting chemicals react with the heme group in haemoglobin present in blood. These chemicals, also known as peroxidase reagents, are colourless dyes that are oxidised to form a coloured product. The reaction between LMG and blood results in a green colour (Farrugia *et al*, 2010).

Research on blood detection in animals that have similarities with reptiles has already been done on fish blood using fluorescein, Bluestar©, phenolphthalein, Hemastix®. The result conclude that fluorescein was found to be the best (i.e., low rate of false positives, detected highest proportion of true positives). Based on this information, fluorescein was investigated further to refine its application in fisheries research. Using fluorescein, injuries could be detected up to 5 hours after the injury occurs and once fluorescein is applied, there is significantly less detectable fluorescein after one hour (Colotelo, 2009). The research on reptil's blood using LMG to

Nureza, F., Putra, B., Yohana, N. and Legowo, D.
Laten Blood Detection Test Sensitifity of Lizard (Varanus salvator) Using Leuco Malachite Green (LMG) with Diffrent Dilution.
DOI: 10.5220/0007542903610364
In *Proceedings of the 2nd International Conference Postgraduate School (ICPS 2018)*, pages 361-364
ISBN: 978-989-758-348-3

see the quality and reaction results has never been done before.

2 MATERIALS AND METHODS

Materials:
LMG, H_20_2, Filter paper, Pipette, Reptile's blood, container, Aquades, EDTA.

Forensic Photography:
Photographs are taken in close-up (photomacrography) of the labeled bloodstain to give an interpretation of the using a Digital Single-lens Reflex (DSLR) Camera and 50mm fixed lens

Sample:
Lizard's blood (*Varanus salvator*) was taken and lied in EDTA tube. Lizard's blood is placed on the ice box and brought to pathology laboratory, Faculty of Veterinary Medicine, Airlangga University.

Blood Dilution:
The blood was diluted with aquadest with dilution 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}.

Figure 1: blood dilution scheme.

Reagen Test and Interpretation

Adapted from Andersson (2017), The LMG solution was prepared by adding 150 ml of aquadest and 100 ml of concentrated acetic acid to 1 g of Leuco Malachite Green into a brown chemical bottle. The solution was stirred until all the Leuco Malachite Green was dissolved and the solution was filtrated. The prepared LMG solution was afterwards transferred from the brown chemical bottle with LMG solution into a smaller separate tube. A 30 % H_20_2 (MERCK®) was diluted with deionised water to a 10 % H_20_2 solution and the H_20_2 was then transferred to a smaller separate tube. A volume of 25 µl of LMG solution and 25 µl of H_20_2 solution was used throughout this study. When not in use, all solutions where kept in a refrigerator.

Methods:
Leuco Malachite Green (LMG) and H_2O_2 were prepared according to Manufacturer guideline and Forensic Laboratory of Indonesian's Police Department as well. All reagents were used according to the manufacturer's guidelines. Positive controls were taken by applying the reagent to a bloodstained piece of filter paper. Negative controls were performed by applying the reagents to a fresh piece of filter paper with no trace of blood.

Sensitivity Testing Autoclaved bottles and distilled H_2O were used. Water was measured using a graduated cylinder and blood was added using a Gilson pipette. Based on Webb *et al* (2006), differing low concentrations of blood were achieved by making a stock solution of blood and distilled water. Solutions of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} were prepared. A set of 35 3cmx3cm pieces of filter paper were placed in each of the diluted blood solutions for each of the presumptive reagents tested. The pieces of filter paper were then removed and allowed to dry for 10 minutes. Each of the pieces of filter paper was then tested with its corresponding reagent to see whether the blood present was detectable. The time taken for the reagent to register a positive result was determined and recorded. Tests were considered negative if reagents failed to react within 4min of exposure to the blood-stained filter paper.

3 RESULT AND DISCUSSION

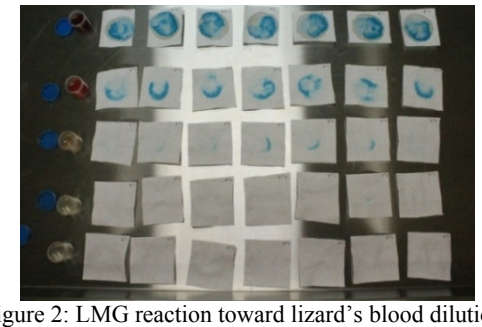

Figure 2: LMG reaction toward lizard's blood dilution.

Sensitivity

Blood Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Lizard	+	+	+	+	-

The table above shows that LMG in lizard's blood can still react to dilution 10^{-4}.

Descriptive

	N	Mean	SD	Min.	Max.
Lizard	35	1.743	1.6688	.0	5.0

Quality
Kruskal-Wallis Test

	Dilution	N	Mean Rank
Lizard	10^{-1}	7	32.00
	10^{-2}	7	25.00
	10^{-3}	7	17.71
	10^{-4}	7	8.29
	10^{-5}	7	7.00
	Total	35	

All the data was analyzed by Kruskal-Wallis test with significancy $p<0.05$ for all groups.

The result of this study shows that different dilution resulted on different interpretation of lizard's blood. Dilution of 10^{-1}, 10^{-2},10^{-3} and 10^{-4} obtained positif (+) result and 10^{-5} obtained negative (-) result.

Hemoglobin concentration can affect the quality of the LMG test reaction. At the higher dilutions it causes a decrease in hemoglobin concentration. Based on Gul and Tosunoglu (2011), the amount of hemoglobin lizard ranges from 8-13 g/dL (Mean: 10.54 g/dL). It is estimated that hemoglobin can no longer be detected by LMG at dilutions of more than 10^{-4}, but can be re-examined in the narrower range between 10^{-4} to 10^{-5}.

4 CONCLUSIONS

The conclusions of this study show that the level of dilution affects the level of sensitivity of LMG test to blood *Varanus salvator*. Sensitivity LMG in lizard's blood can still react to dilution 10^{-4}.

ACKNOWLEDGEMENTS

The author would like to thank Bilqisthi Ari Putra, DVM, Djoko Legowo, DVM and Nadia Yohana, DVM as my partner team for this research.

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