

Quality and Sensitivity of Presumptive Blood Test of Chicken (*Gallus gallus domesticus*) Blood Using Leuco Malachite Green (LMG)

Nadia Yohana¹, Bilqisthi Ari Putra¹, Farah Aidah Nurreza¹ Djoko Legowo²

¹Forensic Science, Postgraduate School, Universitas Airlangga, Jl. Airlangga No. 4-6, Surabaya, East Java, Indonesia 60115

²Veterinary Pathology Department, Faculty of Veterinary Medicine, Universitas Airlangga, C Campus Mulyorejo, Surabaya, East Java, Indonesia 60115

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Abstract: Forensic Science is a branch of medical science that contains the application and utilization of science for law enforcement and justice. Forensic laboratories have the task of supporting a component of case investigation, identifying the case investigation component, the causes of death, known properties and signs for the benefit of proof. The function of blood in the scene of the crime scene is to prove that it is blood of the victim or blood that is not human blood. This research used Leuco Malachite Green (LMG) test. This study aims to determine the quality of LMG reaction in chicken blood with different blood dilution. This research material is LMG solution, chicken blood, aquadest solution. After blood is taken and dilution is performed 10-1 to 10-5. The results of this study were obtained on the dilution of 10-1 obtained positive results (+), dilution 10-2 obtained positive results (+), dilution 10-3 obtained positive results (+), dilution 10-4 and dilution 10-5 obtained negative result (-). The conclusion of this study for the dilution of 10-1 obtained positive results (+), dilution 10-2 obtained positive results (+), dilution 10-3 obtained positive results (+), dilution 10-4 obtained negative results (-), and 10-5 dilution has negative results (-).

1 INTRODUCTION

Forensic Science is a branch of medical science that contains the application and utilization of science for law enforcement and justice (Idries *et al.*, 2011). Blood is probably the most commonly searched body fluid in forensic casework (Takayama, 1912).

The Takayama or Hemochromogen test is used by some forensic laboratories as a confirmatory test for blood. The enhancement reagents often provide valuable aid in crime scene analysis and laboratory examination: in fact, they are able to detect minute traces of blood even when attempts have been made to wash away the incriminating evidence (Takayama, 1912). Leuco Malachite Green (LMG), leuco-crystal violet (LCV), Lumiscene, Bluestar Forensics, Fluorescein, and Luminol are 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. In this case, is the stain found at a crime scene is blood or not (James *et al.*, 2005).

Chicken blood has larger erythrocytes than mammals but it is smaller than mammalian erythrocytes. The erythrocyte size of ungags generally ranges from 10.7 x 6.1 μm to 15.8 x 10.2 μm . In erythrocyte Chicken mature are elliptical shape with a nucleus position in the middle (Bijanti, 2010).

Blood detection in animals in a study similar to that of chicken blood has been performed on fish blood using fluorescein test, Bluestar ©, phenolphthalein, Hemastix®. The conclusion that can be taken that fluorescein found the best results (ie, low false positive rate, detecting the highest proportion of the real positive). So it can be informed, fluorescein testing can be further investigated to improve its application in fisheries research. The use of fluorescein, the injury can be detected up to 5 hours after the injury occurred and the application of once fluorescein, there is fluorescein detected much less after one hour (Colotelo, 2009).

2 MATERIALS AND METHODS

Materials:

Leuco Malachite Green (LMG), H₂O₂, Pipette, Chicken's blood, Ice Container, Aquadest, Ethylene DiamineTetraacetic Acid (EDTA), Syringe, Tube.

Sample:

Chicken's blood (*Gallus gallus domesticus*) taken in a brachial vein with a 3 cc syringe. Blood is inserted in an anti-coagulant tube of Ethylene DiamineTetraacetic Acid (EDTA). Then blood was taken to Pathology Laboratory, Pathology Department, Faculty of Veterinary Medicine, Airlangga University for blood analysis.

Blood Dilution:

The blood was diluted with aquadest with dilution 10⁻¹, 10⁻², 10⁻³, 10⁻⁴ dan 10⁻⁵.

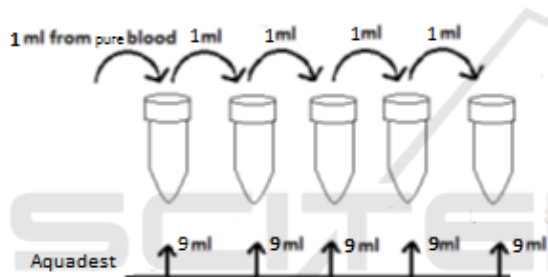


Figure 1: To Study Blood Detection Used Leuco Malachite Green (LMG)

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 % H₂O₂ (MERCK®) was diluted with deionized water to a 10 % H₂O₂ solution and the H₂O₂ was then transferred to a smaller separate tube. A volume of 25 µl of LMG solution and 25 µl of H₂O₂ solution was used throughout this study. When not in use, all solutions where kept in a refrigerator.

Methods:

Leuco Malachite Green (LMG) and H₂O₂ 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 H₂O were used. Water was measured using a graduated cylinder and blood was added using a Gilson pipette. Differing low concentrations of blood were achieved by making a stock solution of blood and distilled water. Solutions of 1:10⁻¹; 1:10⁻²; 1:10⁻³; 1:10⁻⁴; and 1:10⁻⁵ 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 72h. Each of the pieces of filter paper was then tested with its corresponding reagent to see whether the blood present was detectable. The reagents were added directly to the 1 cm² pieces of filter paper. 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

The results of this study were obtained on the dilution of 10⁻¹ obtained positive results (+), dilution 10⁻² obtained positive results (+), dilution 10⁻³ obtained positive results (+), dilution 10⁻⁴ and dilution 10⁻⁵ obtained negative result (-).

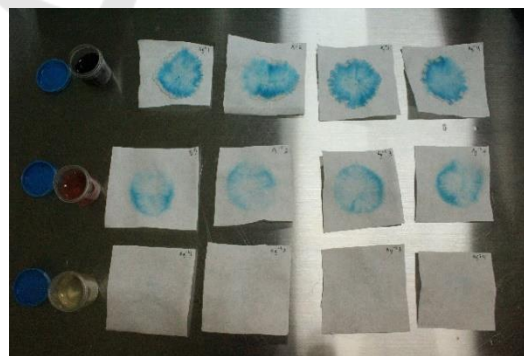


Figure 2: positive results (+) of presumptive test using LMG.

Sensitivity

Blood Dilution	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵
Chicken	+	+	+	-	-

The table above shows that LMG in Chicken’s blood can still react to dilution 10⁻³.

Descriptive

	N	Mean	SD	Min.	Max.
Chicken	35	1.857	1.7846	.0	4.0

Quality

Kruskal-Wallis Test

	Dilution	N	Mean Rank
Chicken	10 ⁻¹	7	29.50
	10 ⁻²	7	27.50
	10 ⁻³	7	18.00
	10 ⁻⁴	7	7.50
	10 ⁻⁵	7	7.50
	Total	35	

All the data were analyzed by Kruskal-Wallis test with significance p<0.05 for all groups. This study resulted that the more dilution of chicken blood, the more negative the LMG test results

The result of this study for the Blood Chicken dilution of 10⁻¹ obtained positive results (+), dilution 10⁻² obtained positive results (+), dilution 10⁻³ obtained positive results (+), dilution 10⁻⁴ obtained negative results (-), and 10⁻⁵ dilution has negative results (-).

Haemoglobin concentration can affect the quality of the LMG test reaction. At higher dilutions it causes a decrease in haemoglobin concentration. Based on Swenson and William (1993), the amount of haemoglobin chicken ranges from 6.50-9.00 g/dL. It is estimated that haemoglobin can no longer be detected by LMG at dilutions of more than 10⁻³, but can be re-examined in the narrower range between 10⁻³ to 10⁻⁵.

4 CONCLUSIONS

The conclusion of this study for sensitivity test and quality test the Blood Chicken dilution of 10⁻¹ obtained positive results (+), dilution 10⁻² obtained positive results (+), dilution 10⁻³ obtained positive results (+), dilution 10⁻⁴ obtained negative results (-), and 10⁻⁵ dilution has negative results (-).

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