



SENSITIVITY AND SPECIFICITY COMPARISON OF PRESUMPTIVE TESTS FOR BLOOD

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Abstract:

Blood is the key evidence in most of the crime scenes which help in establishing the link between the offender and crime scene. For the accurate analysis of suspected blood sample received from the crime scene require a proper collection, packaging and preservation. For the examination of blood, a multistage process is follows in forensic science laboratories. But before going for these steps, presumptive tests are used for preliminary identification of blood. Conventionally Kastle- Meyer and LMG (Leucomalachite Green) tests are used for forensic identification of blood. The aim of this study is to compare the sensitivity and specificity of both Kastle- Meyer and LMG (Leucomalachite Green) tests with accordance to the time of development of color reaction. For this purpose, both fresh and dried blood-stained samples were used to compare the rate of sensitivity of the tests. From this study it is concluded that the sensitivity and specificity of Kastle- Meyer test is more than the LMG (Leucomalachite Green) test for both fresh and dried blood samples. Kastle- Meyer test gives instant results for the blood within 5-20 sec as compare to LMG (Leucomalachite Green) test. Absence of any significant difference between the fresh and dried blood samples for the development of color reaction.

Index Terms: Blood, Presumptive test, Sensitivity, Specificity, Kastle- Meyer and LMG (Leucomalachite Green)

Introduction:

Blood is the main constitute of the human body, which supply nutrients and oxygen to the respective organs of the body. In most of the criminal activities where the physical struggle found between the victim and offender, the possibilities of founding blood on that type of crime scene is maximum [1]. For forensic crime scene expert and serologist, the most important question is to find out that every red color stain is blood or not? [1-3]

To find the answer of this question every forensic expert and serologist goes for the presumptive tests for blood like commonly used tests such as Kastle- Meyer and LMG (Leucomalachite Green) test. Both Kastle- Meyer and LMG (Leucomalachite Green) test works on the enzymatic activity and shows the desired color reaction for the blood pink color in Kastle- Meyer test and green blue color in LMG (Leucomalachite Green) test [1-3].

From many years forensic analysts performing benzidine test for the presumptive analysis. But due to heavy carcinogenic nature of benzidine now most of the forensic lab replaced the benzidine test with Kastle- Meyer test. Which gives better results as the benzidine without any carcinogenic effect in very highly diluted blood samples also [3,4].

The purpose of this study is to compare the time taken by the different samples to develop the color reaction. Which indicate the sensitivity and specificity of the commonly used presumptive tests for identification of blood samples in forensic examination [5-7].

Abbreviations:

KM: Kastle- Meyer test

LMG: Leucomalachite Green test

Research Methodology:

This study focuses on the comparison of the sensitivity and specificity of the Kastle- Meyer test and LMG (Leucomalachite Green) test with accordance to time to develop the color reaction with the blood samples.

For this study below listed samples are analyzed:

Table-1: List of type of samples analyzed

<u>Sr. No.</u>	<u>Age of Sample</u>	<u>Type of Sample</u>
1.	Fresh	Wet Blood
2.	Fresh	Dried Blood
3.	1 Week	Dried Blood
4.	2 Week	Dried Blood
5.	3 Week	Dried Blood
6.	1 Month	Dried Blood

Reagent Preparations:

Kastle- Meyer Test:

- Stock Solution:
 - ✓ Phenolphthalein: 2 gm.
 - ✓ Potassium Hydroxide: 20 gm.
 - ✓ Distilled Water: 100 ml.
 - ✓ Zinc Dust: 20 gm.

Mix, add a few boiling chips and boil under reflux 2-3 hours or until the solution has lost all its pink color.

Cool and decant into a bottle containing some zinc to keep it in the reduced form.

- Working Solutions:
 - ✓ Solution - 1: Ethanol 10 ml.
 - ✓ Solution - 2: Phenolphthalein Stock Solution 2 ml.
 - Distilled Water 10 ml.
 - Ethanol 2 ml.
 - ✓ Solution - 3: 3% Hydrogen Peroxide 10 ml.

(Dilute 30% Hydrogen peroxide to 3% using distilled water)

Procedure:

- ✓ Take the suspected blood sample on the filter paper.
- ✓ Add 2-3 drops of ethanol on sample placed on filter paper.
- ✓ Apply 2-3 drops of working solution.
- ✓ After that note any color change, A pinkish color change at this step indicates a chemical oxidant and the test should be considered inconclusive. If there is no color change, proceed to the next step.
- ✓ Add 2-3 drops of 3% H₂O₂.
- ✓ An immediate pink color change indicates the presence of blood in suspected sample [3,4].

LMG (Leucomalachite Green) Test:

- LMG Reagents:

✓ Leucomalachite Green:	0.25 g
✓ Glacial Acetic Acid:	100 ml
✓ Distilled Water:	150 ml
✓ Zinc Dust:	5 g

Mix, add a few boiling chips and boil under reflux 2-3 hours or until the solution has lost all its color. Cool and decant into a bottle containing some zinc to keep it in the reduced form.

✓ Hydrogen Peroxide:	3%
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Procedure:

- ✓ Take the suspected blood sample on the filter paper.
- ✓ Apply 2-3 drops of LMG reagent.
- ✓ After that note any color change, A blue-green color change at this step indicates a chemical oxidant and the test should be considered inconclusive. If there is no color change, proceed to the next step.
- ✓ Add 2-3 drops of 3% H₂O₂.
- ✓ An immediate green blue color change indicates the presence of blood in suspected sample.

All the samples are procced with the both tests and the time of color change reaction is noted with the help of a stop watch and then analyzed and compare the observation of every sample [3,4].

Results and Discussion:

After analyzing and comparing the samples the following results are observed:

Kastle- Meyer Test:

Kastle Meyer test gives positive result for the all type of blood samples with intense pink color reaction. But the intensity of color and the time taken by the color reaction development is different for every sample. [See Table-2]

Table-2: Color Reaction Sensitivity of Kastle Meyer Test

<u>Sr. No.</u>	<u>Type of Sample</u>	<u>Age of Sample</u>	<u>Color Developed</u>	<u>Time taken to Develop Color</u>
1.	Wet Blood	Fresh	Intense Pink	5 Sec.
2.	Dried Blood	Fresh	Intense Pink	5 Sec.
3.	Dried Blood	1 Week	Intense Pink	5 Sec.
4.	Dried Blood	2 Week	Pink	10 Sec.
5.	Dried Blood	3 Week	Pink	10 Sec.
6.	Dried Blood	1 Month	Pink	15 Sec

LMG (Leucomalachite Green) Test:

LMG (Leucomalachite Green) Test also gives positive color reaction for every blood sample but the intensity of development of color reaction is lower than the comparison of Kastle Meyer test [5-10]. The time of development of color reaction is also different of every sample. [See Table-3]

Table-3: Color Reaction Sensitivity of LMG (Leucomalachite Green) Test

<u>Sr. No.</u>	<u>Type of Sample</u>	<u>Age of Sample</u>	<u>Color Developed</u>	<u>Time taken to Develop Color</u>
7.	Wet Blood	Fresh	Green Blue	50 Sec.
8.	Dried Blood	Fresh	Green Blue	50 Sec.
9.	Dried Blood	1 Week	Green Blue	53 Sec.
10.	Dried Blood	2 Week	Light Green Blue	55 Sec.
11.	Dried Blood	3 Week	Very Light Green Blue	80 Sec.
12.	Dried Blood	1 Month	Very Light Green Blue	120 Sec



KM (Fresh Blood)

Fig: 1- KM test showing Color reaction with Fresh Blood Sample



KM (Dry Blood)

Fig: 2- KM test showing Color reaction with Fresh Dry Blood Sample

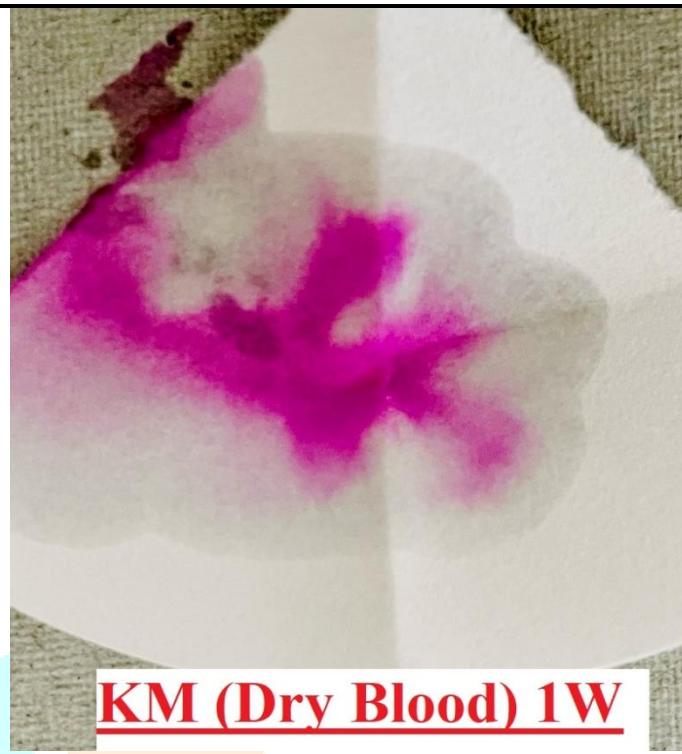


Fig: 3- KM test showing Color reaction with 1-Week-old Dry Blood Sample

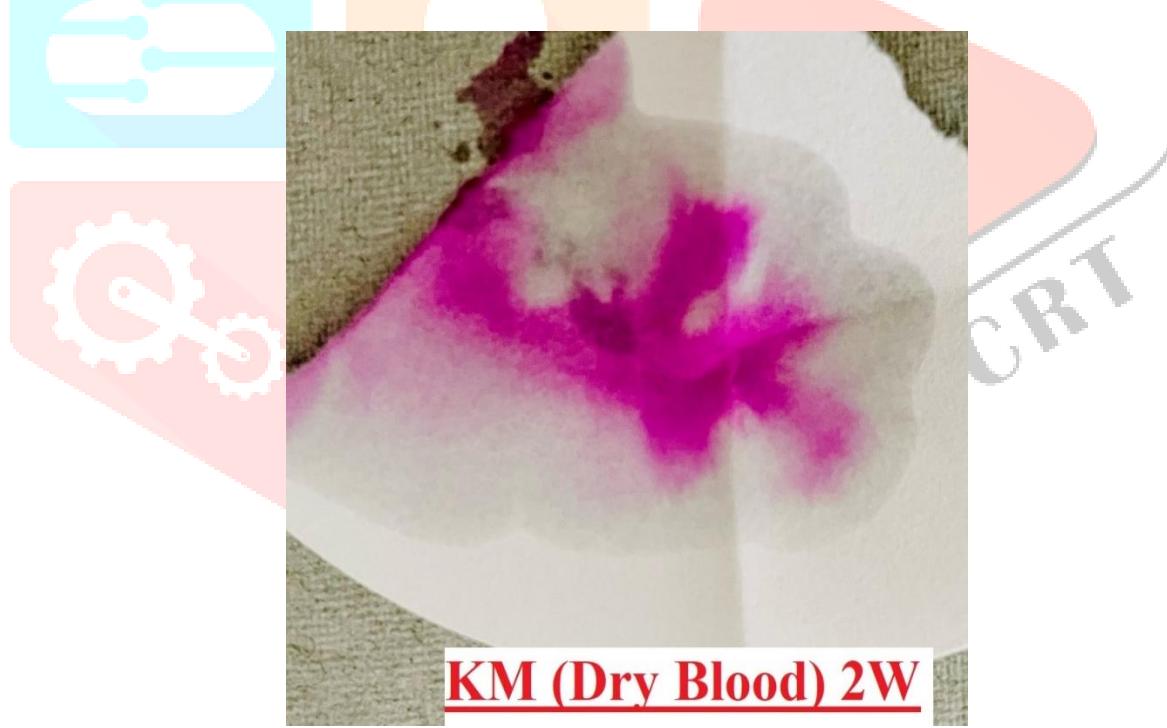


Fig: 4- KM test showing Color reaction with 2-Week-old Dry Blood Sample



Fig: 5- KM test showing Color reaction with 3-Week-old Dry Blood Sample



Fig: 6- KM test showing Color reaction with 1-Month-old Dry Blood Sample

LMG (Leucomalachite Green) Test:



Fig: 7- LMG test showing Color reaction with Fresh Blood Sample



Fig: 8- LMG test showing Color reaction with Fresh Dry Blood Sample



LMG (Dry Blood) 1W

Fig: 9- LMG test showing Color reaction with 1-Week-old Dry Blood Sample



LMG (Dry Blood) 2W

Fig: 10- LMG test showing Color reaction with 2-Week-old Dry Blood Sample



LMG (Dry Blood) 3W

Fig: 11- LMG test showing Color reaction with 3-Week-old Dry Blood Sample



Fig: 12- LMG test showing Color reaction with 1-Month-old Dry Blood Sample

After analyzing fresh and dried blood samples with KM and LMG reagents it seems that Kastle Meyer test shows more sensitive and specific than the LMG test [5-10]. In Kastle Meyer test there is no significant variations are shown in results with fresh and dried blood stains but the intensity of color reaction changes

from intense pink to pinkish for 3 weeks and 1 month old dried blood stains [7-9]. Fresh dried and wet blood samples take the same time 5sec. to show the positive results for KM test [5-7].

In case of LMG test dry and fresh blood stain takes same time 50 sec to develop positive color reaction. But for other samples 1-week, 2-week, 3-week and 1-month old the color of positive reaction gradually changes from green blue to light green blue as the sample getting old with the time [5-7].

For all the blood samples KM test takes 5 to 15 sec but in LMG test it takes 50 to 120 sec to develop color reaction. But the intensity of color changes dark to light.

Table-4: Sensitivity Comparison between Kastle Meyer and LMG (Leucomalachite Green) Test

<u>Sr. No.</u>	<u>Type of Sample</u>	<u>Age of Sample</u>	<u>Time taken to Develop Color</u>	
			<u>KM Test</u>	<u>LMG Test</u>
1.	Wet Blood	Fresh	5 Sec.	50 Sec.
2.	Dried Blood	Fresh	5 Sec.	50 Sec.
3.	Dried Blood	1 Week	5 Sec.	53 Sec.
4.	Dried Blood	2 Week	10 Sec.	55 Sec.
5.	Dried Blood	3 Week	10 Sec.	80 Sec.
6.	Dried Blood	1 Month	15 Sec	120 Sec

Conclusion:

From this study of presumptive tests for blood we can conclude that the Kastle Meyer test is more specific and sensitive than the LMG (Leucomalachite Green) Test. Kastle Meyer test takes less time to develop positive color reaction than the LMG (Leucomalachite Green) Test. It is also concluded that the there is no significant difference found between fresh and dry blood samples to develop positive color test. So, the Kastle Meyer test is the real replacement of benzidine tests for the identification of blood in forensic science labs.

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