

ESTIMATING THE NEGATING EFFECT OF COMMON CONTAMINANTS ENCOUNTERED IN INDIA ON PRESUMPTIVE FORENSIC TESTS FOR BLOOD

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Abstract

Blood is the most common evidence encountered in crimes and is many a times crucial in fixing the corpus delicti, proving the modus operandi and also the actus reus during a crime. Presumptive testing of suspected blood stains is the first step in forensic examination of blood. Presumptive tests are usually performed at the scene of crime and forms the basis on which a stain is collected for further examination in the FSL. The common presumptive tests performed for identification of blood are Benzidine and Kastle Mayer tests. Even though the efficacy of presumptive tests is highly reliable, in presence of certain contaminants their efficacy is altered. In South India common practise of using beautifying skin and hair additives is predominantly observed. This study attempts to identify the extent to which common contaminants namely turmeric, kumkum and coconut oil reduce the efficacy of the presumptive tests for blood.

Keywords: Forensic Science, Forensic Biology, Presumptive tests, Benzidine, Kastle Mayer, Phenolphthalein, Turmeric, Kumkum, Coconut oil, Contamination.

INTRODUCTION

Biological evidence can be expected in any crime that involves human beings. In investigation of violent crimes, examination of biological materials becomes very important as it can link a suspect to the victim or the scene of crime (Locard's Principle of Exchange). Careful collection, proper packaging and preservation of biological evidence should be done as they deteriorate due to microbial growth and environmental conditions. Failure in doing so can result in loss of evidentiary value. The evidence is analysed and the forensic biologist establishes their relation with the crime in their expert testimony. (Justus & B, 2006; Singh, 2021)

Blood as evidence is encountered in cases of murder, dacoity, physical assault, rape, hurt and other criminal offences. It helps prove that an incident of violence has taken place (corpus delicti), helps identify species of origin (whether it is human or non-human blood), and also helps determine the blood group. DNA analysis is also possible with blood evidence. (Sharma et al., 2019)

A visual observation of the questioned stain, followed by highly sensitive presumptive tests done at the scene of crime and then moving onto highly specific confirmatory tests performed in the lab, provides sound data to support identification of blood. (James et al., 2005)

Presumptive tests are those which when positive for blood produce a visible colour reaction or those that result in a release of light. This allows the examiner to make a qualitative conclusion that blood is present in the sample that was tested. When negative, these tests help to eliminate stains that need no further consideration. Once positive, the remaining sample if present is collected and further testing is done in the lab to confirm the presence of blood as no single presumptive test is absolutely specific for blood. Oxidation - reduction is the chemistry involved in the presumptive tests. These reactions are catalysed by Heme, a component of haemoglobin which is a colourless substrate that undergoes oxidation-reduction resulting in the change of colour or fluorescence. The most common presumptive tests used for forensic identification of blood are Benzidine and Kastle Mayer tests. (James et al., 2005)

Blood evidence is not always found in its pure state as potential contamination can occur at the crime scene, during the packaging, collection and transportation to laboratory, or during analysis and storage. So, these contaminants when mixed with blood evidence can cause some interference with results of presumptive tests as they lack efficacy. Contaminants like rust, red oxide, vegetable stains can have a direct effect on the quality of forensic examination that occurs at the scene. Other sources of contamination can be introduced by the person from the usage of beauty products or something that the person has handled before the blood evidence was created. People in south India incline towards the use of natural products in their day to day lives. In the recent past the use of spices being sprayed over the crime scenes in an effort to cover up the crime is on the rise. In this

study, attempts have been made to identify the effects on presumptive tests by the common products which may be encountered as contaminants - turmeric, kumkum and coconut oil. (Ashok Babu & Venkateswaran K, 2016; Gopinath & Karthikeyan, 2018; Pandiselvam et al., 2019)

Kasthuri Bai et al, 2007 showed that turmeric caused interference in phenolphthalein test in a trap case. Sodium carbonate hand washings of the accused showed a reddish yellow colour because of the turmeric coating present on the hands of the accused instead of the pink colour of phenolphthalein. Curcumin is a pigment in turmeric which imparts yellow colour and its presence in alkaline medium gives brownish-red colour (polyphenolic compound). Hence the higher concentration of turmeric masked the normal pink colour of phenolphthalein. (Kasthuri bai et al., 2007)

Fukushima et al, 2019 tested the sensitivity of Kastle-Meyer test by using blood dilutions 1:10, 1:100, 1:1000, 1:10,000, 1:100,000 and 1:1,000,000. It was found that it was able to detect human blood up to 1:10,000 dilution and beyond this dilution no colour change was observed. Hence, this test was considered a good presumptive test as it was able to detect blood at different dilutions. (Fukushima et al., 2019)

Tobe et al, 2007 tested the sensitivity of presumptive tests for blood using blood dilutions 1:10,100, 1:100,000, 1:1,000,000, 1:5,000,000 and 1:10,000,000. Luminol was found to have the greatest sensitivity. It was able to detect blood for both 1:10,000 and 1:100,000 dilutions and beyond this dilution, no reaction was observed within 4 minutes. The chemiluminescence seen for both dilutions lasted for about a minute but it was less intense than the positive reaction for actual blood. Leucomalachite green test was found to have ten times lesser sensitivity. It was able to detect blood at 1:10,000 dilution and beyond this dilution, no colour change was observed within 4 minutes. Kastle Meyer test was found to have the same sensitivity as that of other tests. It was able to detect blood at 1:10,000 dilution for all samples and at 1:100,000 dilution for 3 out of 25 samples. Beyond this dilution, no colour change was observed within 4 minutes. (Tobe et al., 2007)

Cox M, 1991 made a comparative study on the sensitivity of presumptive tests which included phenolphthalein, tetramethylbenzidine, leucomalachite green and ortho-tolidine tests using blood dilutions 1:50, 1:100, 1:500, 1:1000, 1:5000, 1:10,000, 1:50,000, 1:100,000, 1:500,000, 1:1,000,000 and 1:2,000,000. Phenolphthalein test was able to detect blood up to 1:1,000,000 dilution for blood solution, while on filter paper and cotton cloth, it was able to detect blood up to 1:10,000 dilution. Benzidine test was able to detect blood up to 1:1,000,000 dilution on filter paper, cotton cloth and blood solution. (Cox, 1991)

AIM OF THE STUDY

To identify if existing presumptive tests for forensic identification of blood are impacted by contaminants - turmeric, kumkum and coconut oil prevalently used in south India.

SAMPLE

The contaminants were purchased from the retail stores and the most popular brands for each of these contaminants were chosen

- Turmeric - Eastern
- Kumkum - Gopuram
- Coconut Oil - Parachute

CONCEPTUALISATION

Attempts were made to test contaminated blood in increasing concentrations of the 3 different contaminants. The threshold concentration at which each contaminant gave a false negative result for each presumptive test was identified.

Stock contaminant solutions were prepared at 4g% by dissolving in distilled water.

Blood for testing was prepared by diluting 100µL in 100ml of normal saline (0.85% sodium chloride in distilled water).

Six watch glasses were used to perform the serial dilutions.

In each watch glass 100µL of normal saline was added. To the first watch glass 100µL of diluted blood was added and mixed thoroughly. 100µL of the mixture in the first watch glass was added to the second watch glass and mixed thoroughly. Similarly, 100µL of mixture was removed from a watch glass and added to the next watch glass and mixed thoroughly. The 100µL mixture from the last watch glass was discarded. This enabled a serial dilution of the diluted blood sample in the following concentrations.

100µL of stock contaminant solution was added to each watch glass containing serially diluted blood.

Watch Glass	Concentration of blood +	Concentration of blood +	Concentration of blood +
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	Turmeric contaminant	Kumkum contaminant	Coconut oil contaminant
1st dilution	Blood - 0.05 ml% Contaminant - 4g%	Blood - 0.05 ml% Contaminant - 4g%	Blood - 0.05 ml% Contaminant - 4ml%
2nd dilution	Blood - 0.025 ml% Contaminant - 4g%	Blood - 0.025 ml% Contaminant - 4g%	Blood - 0.025 ml% Contaminant - 4ml%
3rd dilution	Blood - 0.0125 ml% Contaminant - 4g%	Blood - 0.0125 ml% Contaminant - 4g%	Blood - 0.0125 ml% Contaminant - 4ml%
4th dilution	Blood - 0.00625 ml% Contaminant - 4g%	Blood - 0.00625 ml% Contaminant - 4g%	Blood - 0.00625 ml% Contaminant - 4ml%
5th dilution	Blood - 0.00313 ml% Contaminant - 4g%	Blood - 0.00313 ml% Contaminant - 4g%	Blood - 0.00313 ml% Contaminant - 4ml%
6th dilution	Blood - 0.00156 ml% Contaminant - 4g%	Blood - 0.00156 ml% Contaminant - 4g%	Blood - 0.00156 ml% Contaminant - 4ml%

Table 1. Serially diluted blood with increasing concentration of each contaminant.

The above was performed in two sets to enable both Benzidine and Kastle Meyer tests to be performed. To the first set, Benzidine test was performed and to the second set Kastle Meyer test was performed separately.

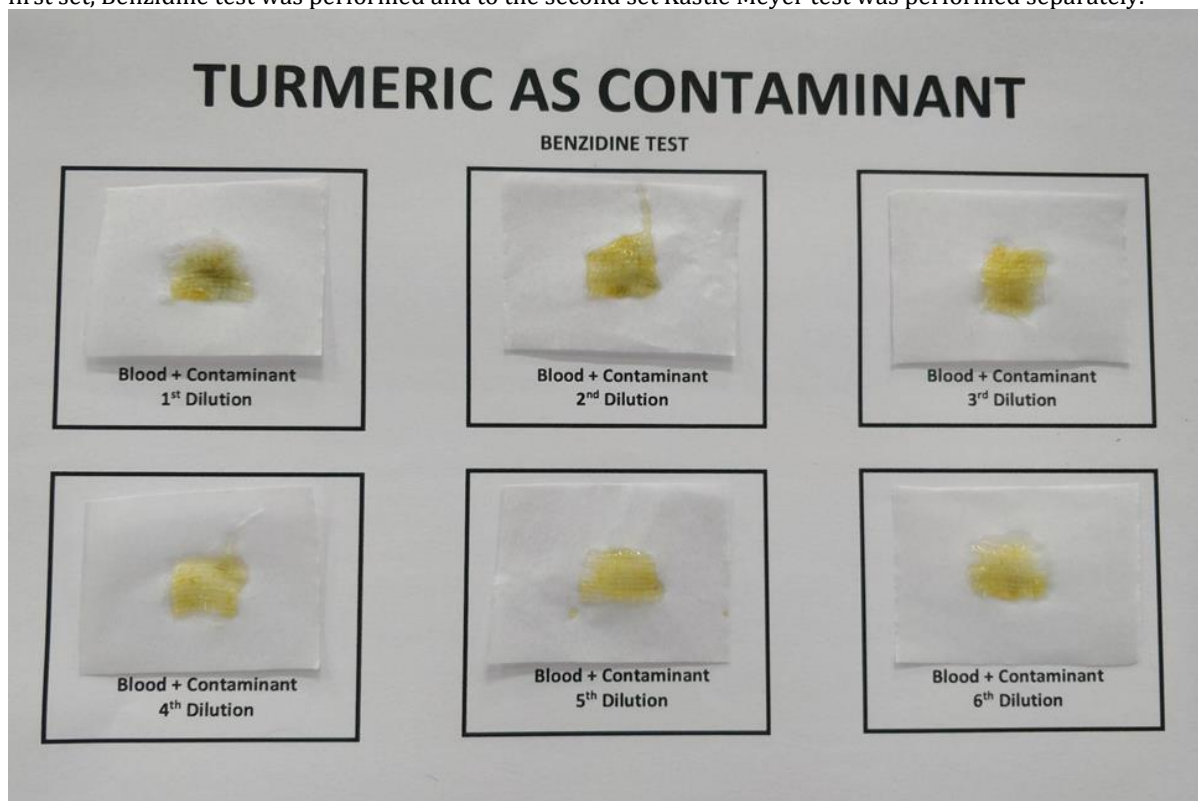


Figure 1. Benzidine test on blood with turmeric as contaminant.

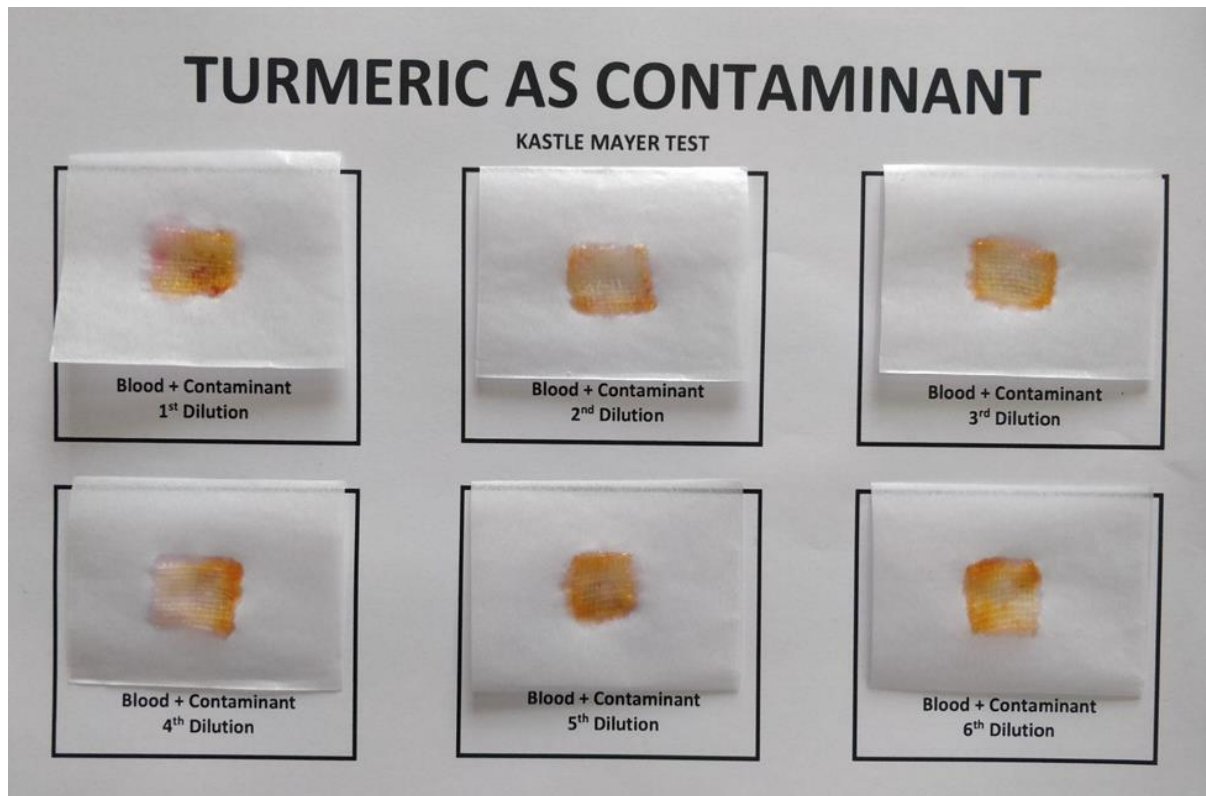


Figure 2. Kastle Mayer test on blood with turmeric as contaminant.

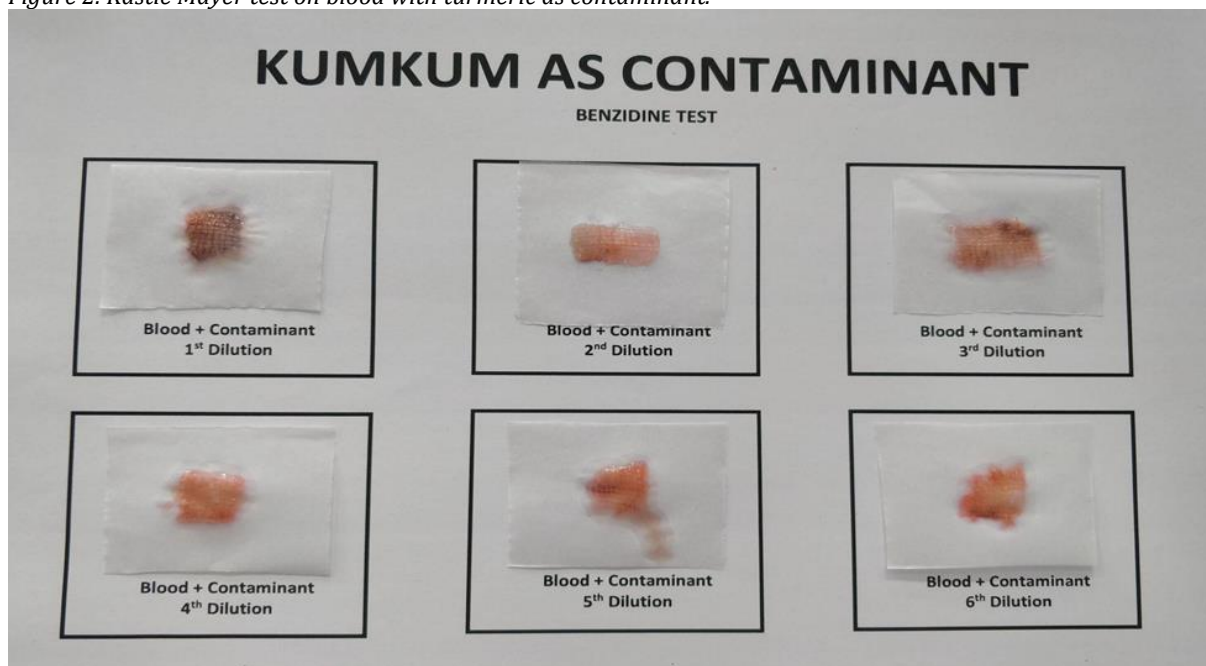


Figure 3. Benzidine test on blood with kumkum as contaminant.

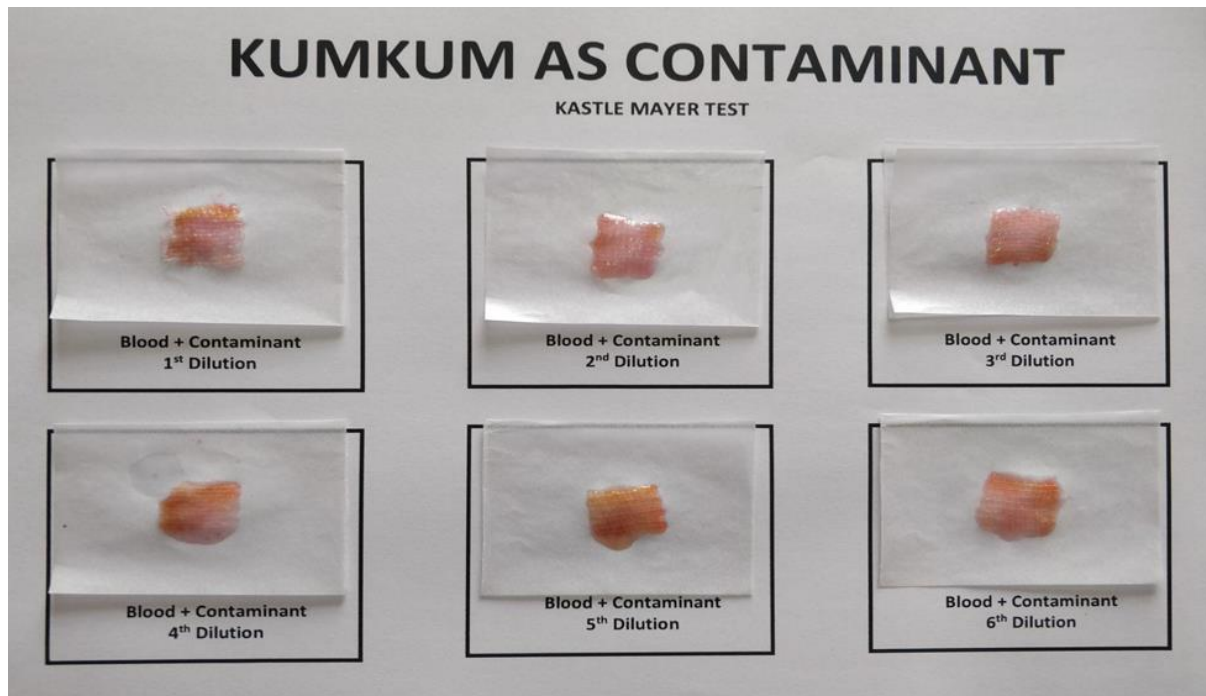


Figure 4. Kastle Mayer test on blood with kumkum as contaminant.

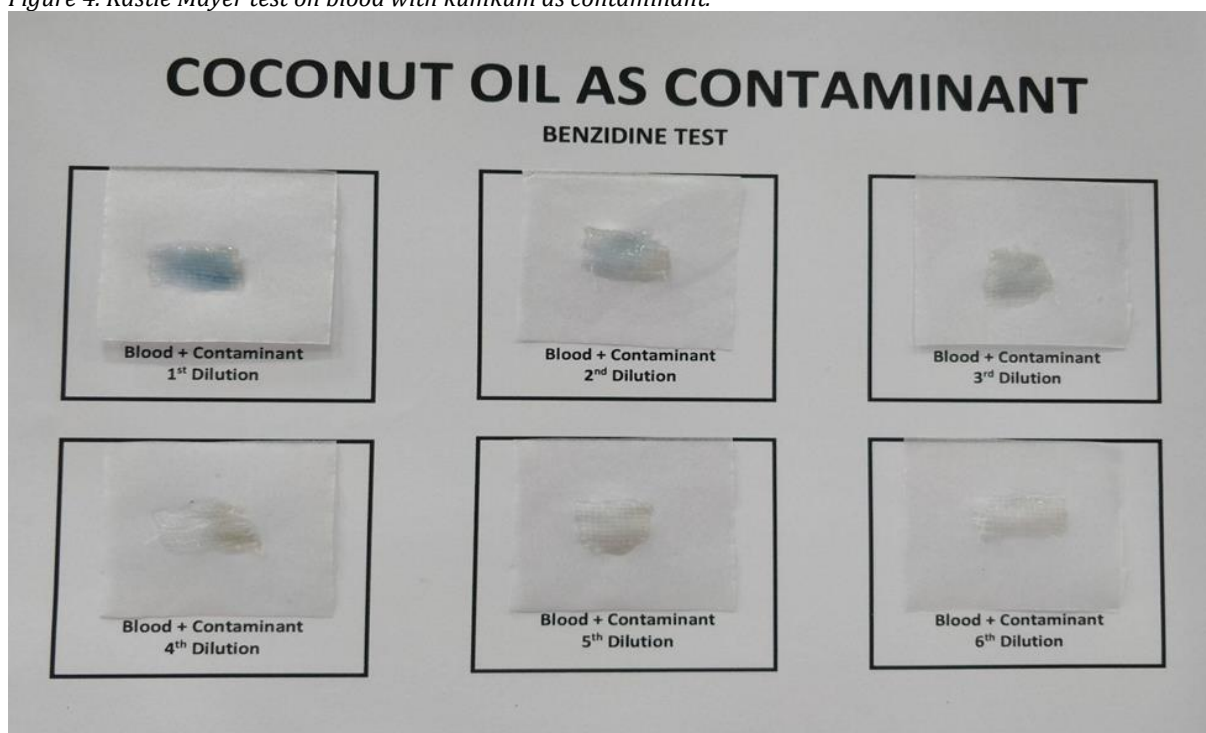


Figure 5. Benzidine test on blood with coconut oil as contaminant.

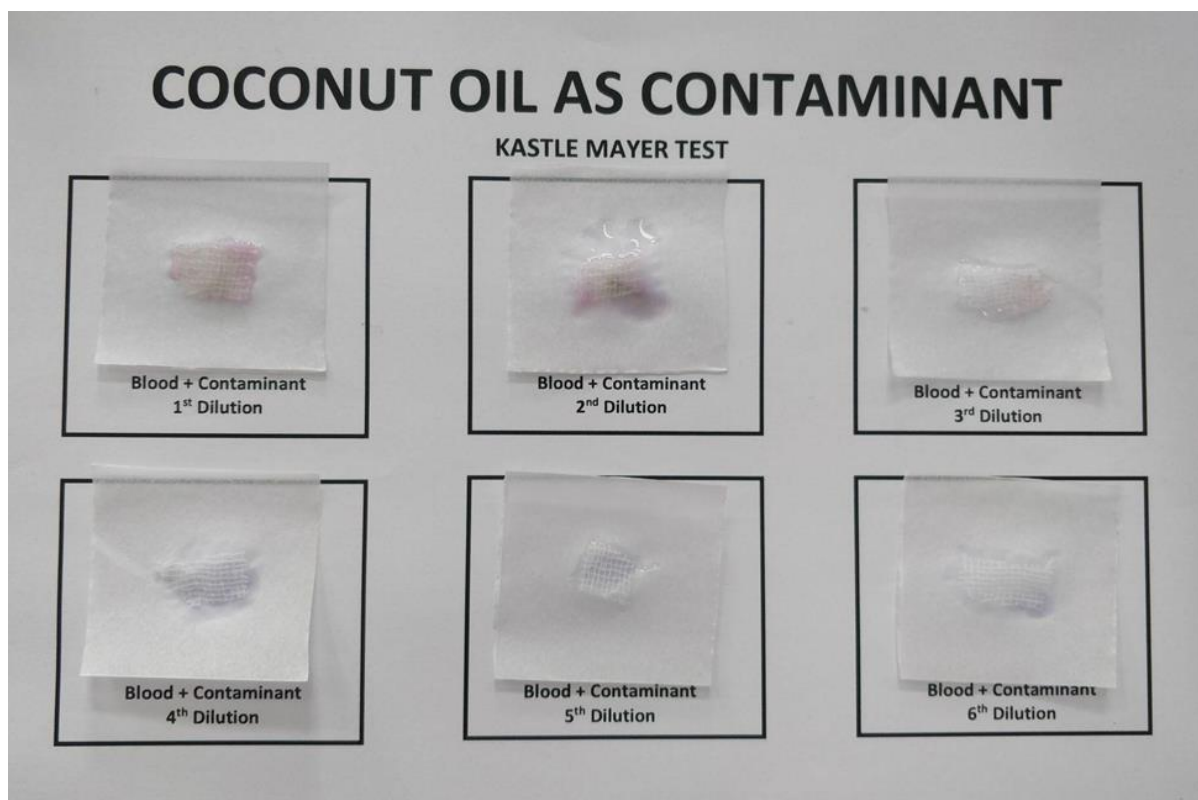


Figure 6. Kastle Mayer test on blood with coconut oil as contaminant.

Concentration	Turmeric		Kumkum		Coconut oil	
	Benzidine test	Kastle-Meyer test	Benzidine test	Kastle-Meyer test	Benzidine test	Kastle-Meyer test
Blood – 0.05 ml% Contaminant – 4g%	Blue green colour	Faint pink colour	Blue green colour	Faint pink colour	Blue Green colour	Faint pink colour
Blood – 0.025 ml% Contaminant – 4g%	No colour change	Faint pink colour	No colour change	Faint pink colour	Blue Green colour	Faint pink colour
Blood – 0.0125 ml% Contaminant – 4g%	No colour change	Faint pink colour	No colour change	Faint pink colour	Faint Blue green colour	No colour change
Blood – 0.00625 ml% Contaminant – 4g%	No colour change	Faint pink colour	No colour change	Faint pink colour	No colour change	No colour change
Blood – 0.00313 ml% Contaminant – 4g%	No colour change	Faint pink colour	No colour change	Faint pink colour	No colour change	No colour change
Blood – 0.00156 ml% Contaminant – 4g%	No colour change	Faint pink colour	No colour change	Faint pink colour	No colour change	No colour change

Table 2. Results of conceptualisation.

Challenges identified at the end of conceptualisation

1. Kastle Mayer test could not be performed in the case of turmeric and kumkum for this study because of the interference of the pink colour in presence of turmeric and kumkum (false positive results were noted in Kastle Meyer test in presence of trace amounts of turmeric and kumkum. This was in consonance with the literature cited. (Kasthuri bai et al., 2007) So, it was decided to discontinue performing the Kastle Mayer test for turmeric and kumkum as contaminants.
2. However, in the case of coconut oil, both Benzidine and Kastle Mayer tests were continued to be performed.

Replication

The last dilution at which each contaminant gave a false negative to contaminated blood was repeated 30 times each. This was performed for each Benzidine and Kastle Mayer test separately for coconut oil and only Benzidine test was performed in the case of turmeric and kumkum. The replication was performed to ensure the accuracy of findings of the conceptualisation phase.

The findings of the replication phase also was in consonance with the conceptualisation phase.

Control Validation

Diluted blood without addition of contamination was tested 10 times to ensure the diluted blood's positivity to the presumptive tests and it was found to be positive.

FINDINGS

Contaminants addition was certainly reducing the efficiency of the presumptive tests - Benzidine and Kastle Mayer tests in the different contaminants that were used.

In case of Turmeric and Kumkum as contaminants, it was found that the benzidine test did not detect presence of blood in a dilution mixture of Blood - 0.025 ml% + Contaminant - 4g% and above. The Kastle Meyer test for turmeric and kumkum could not be interpreted due to the interference of turmeric and kumkum with phenolphthalein. For coconut oil, it was found that the Benzidine test did not detect presence of blood in a dilution mixture of Blood - 0.00625 ml% + Contaminant - 4ml% and above. The Kastle Mayer test did not detect presence of blood in a dilution mixture of Blood - 0.00125 ml% + Contaminant - 4ml% and above.

DISCUSSION

The findings of this study bears weightage in forensic analysis at the scene of crime. The presumptive tests - Benzidine and Kastle Mayer are the most commonly performed presumptive tests and the results of these tests form the decision to whether the evidence is collected or not for further analysis. Usage of turmeric, kumkum and coconut oil is very prevalent in many regions of the world, especially in south India. They may be used as condiments or cosmetics by the victim, accused, bystanders, first responding officers etc. and can pose to be a contaminant. The use of spices to coverup the crime by criminals is a common practice in recent times which again poses these contaminants in the scene of crime.

This study highlights the need to remain vigilant during analysis at the scene of crime and to exercise caution during use of presumptive tests. As demonstrated, the extent of inefficacy caused by the different contaminants are varying to each other but there is a negating effect on the presumptive tests which needs to be considered. Undue relying on presumptive tests must be addressed.

CONCLUSION

In cases where scientific evidence was heavily relied on, false positive / false negative results can be of major concern. They can lead to a rampant rate of acquittal.

Presumptive tests are relied on by field scientific officers to decide whether the evidence needs to be collected and forwarded to the Forensic Science Laboratories. When these tests provide false positive / false negative results, this can adversely affect the progress of the investigation.

This study addresses the extent to which common contaminants can lead to false interpretation of the commonly used presumptive tests.

Based on the above findings, the study found that contaminants reduced the efficiency of Benzidine and Kastle Meyer Tests. Turmeric and kumkum caused greater interference whereas Coconut oil caused the least interference. Turmeric and kumkum caused false positive results when tested with Kastle-Meyer Test. Kastle Meyer test showed lesser sensitivity than Benzidine Test when coconut oil was used as contaminant.

The contaminants used in this study are limited to Indian context and are not widely popular in other international geographic locations. Since it was a pilot study, only three contaminants (turmeric, Kumkum, and coconut oil) were used to study their effect on the efficacy of presumptive tests.

The study can form a basis by providing preliminary findings for future analysis on interference of contaminants on different presumptive tests of blood. This study can be broadened to identify if similar contamination is possible even in other parts of India with the same contaminants or with different contaminants, if so, to identify possible lists of contaminants and their impact of contamination.

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