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Research Article

Evaluating Benzidine for Rapid Detection of Blood Remnants on Fabrics after Multiple Washing Cycle

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ABSTRACT

Blood is the most frequent type of evidence used in criminal cases, commonly used to prove the modus operandi, actus reus, and corpus delicti of a crime. The initial step in a forensic investigation of blood is the presumptive inspection of likely blood stains. Presumptive tests are typically performed at the crime site and serve as the basis for further investigation. Testing with benzidine is a common presumptive technique used for detecting blood. Since Criminals frequently use a variety of techniques in order to cover up or entirely remove blood stains from their clothing or other materials. Therefore, this study aims to investigate the effectiveness of the Benzidine Test in identifying dried bloodstains on different types of fabrics after multiple washing cycles and also determines how detergents and washing techniques affect the fabric's ability to retain bloodstains.

KEYWORDS: Benzidine test, Washing, fabrics, Temperature, hygiene, health, bloodstain

1. INTRODUCTION

Benzidine test, Washing, fabrics, Temperature, hygiene, health, bloodstain. Forensic Biology involves the morphological examination of hair samples, fibers, wood fragments, seeds, leaf fragments, pollen, micro flora, etc. generated from the crime scene and the victims' bodies. It also involves bacteriological and entomological examinations. [1] Forensic serology refers to the analysis of blood, semen, saliva, etc., and its nature, origin, and grouping from the crime scene. One of the subfields of Forensic Biology includes Forensic serology which is an important element of modern forensic science. The scientific foundations of forensic biology were established in 1853 when the Polish anatomist and doctor Karol Teichman Stawiarski developed a test that allows the identification of brown stains found on clothes. As comes from blood. Later, other chemical methods for the detection of blood like Kastle-Meyer (KM), benzidine and Luminol were also created. In 1901, Paul Uhlenhuth developed a method to separate human blood from animal blood for micro evidence. The dynamic development in this field of forensic medicine began with Karl Landsteiner's discovery of blood groups (ABO). In1901, Max Richter presented the first studies on the differentiation of blood groups in blood stains, work that led to the creation of the method in 1916 by Leon Latte to determine the blood group in blood spots. In the following years, this method was improved by Józef Holzer. [1] The main task of forensic serologists is to identify body fluids. Liquid stains found on the scene of the crime are usually associated with violent crimes. Evidence of the presence of bodily fluids can confirm alleged acts of violence for investigative purposes. [1]

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Blood is the most commonly collected and one of the most important pieces of evidence used in forensic investigations. It is usually obtained in connection with murders, assaults, and terrorist attacks, including bombings. Blood as evidence is important in the criminal justice system because it can link a crime to a criminal or rule out an individual's involvement in a crime. In addition, the pattern of bloodstains can aid in crime scene reconstruction by providing information about the relative location and movement of the offender and victim within the crime scene. [2]

Blood is a viscous fluid connective tissue that is made up of cellular elements suspended in plasma. Plasma is a viscous, translucent, yellowish fluid composed of water (90%), proteins (7%), organic salts (1%), and organic compounds (2%), such as amino acids, lipids, and vitamins. The total volume of human blood is about 5 liters (depending on the size of the body). Outside the blood vessels, a complex reaction takes place in the blood called coagulation or thrombus formation, which plays an important role in repairing damaged blood vessels and preventing blood loss [2] other components of blood include erythrocytes (red blood cells), leukocytes (white blood cells), and thrombocytes(platelets). Blood is the most frequently retrieved and vital kind of evidence employed in forensic investigations. It is more often recovered in cases of homicide, assault, and terrorist attacks, including bombings. Blood as evidence is important in the criminal justice system because it can connect a crime to a criminal or rule out an individual's involvement in a crime. Furthermore, the pattern of blood stains can aid in the reconstruction of a crime scene by transmitting information about the criminal's and victim's relative position and movement at the crime scene.

Presumptive and confirmatory test

It is critical to determine the nature, origin, and other components of the blood/blood stain retrieved from the crime scene. Chemical examinations are divided into two broader categories; Preliminary tests and Confirmatory tests. Presumptive tests may indicate the likelihood that a particular physiological tissue or fluid is present, the purpose of preliminary investigations is to check if the liquid found is blood and if its origin is human. Although a detailed examination of suspected blood colour requires analytical laboratory equipment and technical expertise. This examination is called a presumptive blood test, where it rules out the possibility that fluid is blood. [3] In numerous cases, the discovery of blood is the first step for further forensic analysis, similar to DNA (deoxyribonucleic acid) profiling, which provides the pivotal substantiation of individualization or when the suspected bloodstains have been washed either with water or with soap/detergents. whereas a confirmatory test is subsequently carried out to be able to identify a specific biological material. Confirmatory tests offer more trustworthy evidence and are crucial in court cases. To achieve accurate results, it is crucial to work with forensic specialists and adhere to accepted laboratory procedures when conducting these tests. [4]

Tetramethyl Benzidine

The presumptive blood test for tetramethylbenzidine (TMB) is a catalytic test that is based on hemoglobin peroxidase-like activity. Hemoglobin has the ability to cleave oxygen molecules of H2O2(hydrogen Peroxide) and catalyze the reaction of the reduced form of 3,3',5,5'-tetramethylbenzidine to the oxidized blue-green product. Hemoglobin in the blood contains iron and is responsible for the red color of the blood. [5,7] The hemoglobin in our blood has peroxidase-like activity, which means it can catalyze (speed up) break down peroxide, and release an oxygen molecule. It is a sensitive test to detect the presence of blood based on the development of blue color in contact with benzidine, hydrogen peroxide, and glacial acetic acid. TMB is used in acidic media when given as a solution and results in a positive green to blue-green color. Care must be taken not to add too much reagent to the blood stain as this will turn the reaction dark blue and may cloud the brush. False positives can be seen with substances that have been pretreated with some cosmetics since 1904, benzidine has been extremely popular as well as reliable. It was found to be a sensitive and specific test for blood. Over time, benzidine was found to be nonspecific for blood, but specific for peroxidase [8,9,11]

Absorbent Fabrics and non-Absorbent Fabrics

Absorbent fabric refers to a textile material that can absorb and retain moisture or liquid. They are usually made from natural fibres such as cotton, bamboo or linen. These fibres have a large surface area and capillary action that allows them to move moisture away from the surface and retain it in the fibres. For this study, cotton fabric was used. [10,11]

Non-absorbent fabric refers to a textile material that cannot absorb or retain moisture or liquid. Non-absorbent fabrics are usually made from synthetic fibres such as nylon, polyester or polypropylene. For this study, the polyester fabric was used. [10,11]

Blood evidence found at a crime scene can be important evidence in a criminal investigation. It provides valuable information regarding the identity of the victim and possible suspects, as well as how the crime was committed. Criminals often try to remove blood stains which happen to come in contact with the fabric they wore during committing the crime. They try different methods to clean the blood stains without leaving any traces on them like washing with different detergents [7], washing with water of different temperatures, etc. These methods can lead to modification and partial or complete removal of the discolored areas. This study aims to examine the efficacy of the TMB test in identifying dried blood stains on various types of fabrics after multiple washing cycles. Additionally, it seeks to investigate how the use of detergents and varying washing techniques and immersion time impact the ability of fabrics to retain blood stains. The study design adopted is exploratory and experimental. In actuality, this discovery is crucial because it can help criminal investigators establish if a specific fabric was used in a crime or

not, based on the fabric's tendency to preserve blood stains even after several washes, both using and without detergents [7]. Furthermore, the dissertation can add to the current volume of evidence on the effects of TMB on absorbent and non-absorbent fabric materials, as well as the role of other elements in the study. Such information could be beneficial for future research in this field, as well as forensic investigations and criminal justice decisions.

Health risks of residual blood harboring pathogens/infectious agents

Health risks of residual blood harboring pathogens/infectious agents. The practice of hospital staff members washing their lab coats, Uniforms, and operating room scrubs at their residents to reduce expenses raises concerns about escalated rates of hospital-acquired infections and further has the potential of environmental dissemination of infectious agents. Hospital textiles generally include bed linens, blankets, towels, personal clothing items of patients and personnel, and attire for surgical procedures such as gowns and drapes, which may serve as vectors for the transmission of pathogens if not properly decontaminated through rigorous laundering protocols. [14] Based on some studies Fabrics often contain high numbers of microorganisms from various body tissues and fluids such as blood, skin, stool, urine, Vomitus, etc. Generally, the purpose of cleaning revolves around two factors, first to restore the appearance of cloth and prevent its deterioration and second, to reduce the number of microorganisms along with any substances supporting their growth. [15] Microorganisms found commonly on hospital textiles are: Gram-negative bacteria, coagulase-negative staphylococci, *Bacillus* sp., and typical skin flora [16]. Some people working in health care assume that visibly cleaner garments are safer for them to use, but remain far from sterilization and encourage infections or diseases. A study reported that bacterial organisms like enterococci, Enterococcus faecium, Staphylococcus aureus, Enterobacter aerogenes, and Pseudomonas aeruginosa can survive laundering temperatures up to 60°C, not at 75°C. [18] However, some studies have shown that using detergents and disinfectants that are high-tech can achieve disinfection even at 30°C for Enterococcus faecium and Enterobacter aerogenes. One study identified the optimum laundering temperature for disinfection as 40°C when proper procedures are followed. [17]

2. METHODS

Collection of samples

Blood samples were taken from the blood bank, preserved in an anti-coagulant EDTA (ethylenediaminetetraacetic acid.) tube, and put in the refrigerator for later use. The cut pieces of the absorbent fabrics (6x6cm) were pinned onto a thermocouple board. Control positive and negative samples were also attached in parallel to the test samples and 2ml of blood was then added to each fabric carefully. Once the blood spots were made, the fabrics were then allowed to dry at room temperature for 24 hours before proceeding to the first wash. The cut pieces of the non-absorbent fabrics (6x6cm) were also attached to a thermocol board. 29 Control positive and negative samples were also attached in parallel to the test samples and 2 ml of blood was then added to each fabric carefully. Once the blood spots were made, the fabrics were then allowed to dry at room temperature for 24 hours before proceeding to the first wash. [13]

Note: For control negative samples, red food colouring was used. This was previously tested under the benzidine test and produced negative results.

Figure-1: Sample Preparation for absorbent fabrics (cotton)

Tetra Methyl Benzidine (TMB) Preparation

To prepare the solution, 0.25 grams of Benzidine powder was carefully measured and combined with 0.10 milliliters of 30% Hydrogen Peroxide (AR) and 2.16 milliliters of Glacial Acetic Acid. This mixture was gently warmed to facilitate dissolution and homogenization. Subsequently, 10 milliliters of 0.85% Normal Saline Solution and 5 milliliters of Distilled Water were added to the Benzidine mixture. After the addition of the Distilled Water, the solution was briskly shaken to ensure thorough mixing and homogeneity. This method yields a solution containing Benzidine, suitable for its intended application in subsequent procedures or analyses. [12]

Washing Methodology

The washing methodology involves washing of stained fabrics using different conditions and temperatures, followed by testing with the Benzidine test to detect blood. The first set of washings occurs at a room temperature, both with and without detergent, using a 500ml beaker. The second set involves washing at 50°Celsius, again with and without detergent. Lastly, the fabrics are washed at 100°Celsius, with and without detergent. In each case, the stained fabrics were immersed or soaked for a specific duration, followed by hand washing to remove bloodstains.

Positive and negative controls are washed alongside the stained fabrics in parallel. After washing, the fabrics were allowed to dry at room temperature after washing and then tested with the Benzidine, where color changes are noted to determine the presence of traces of blood. Through these variations in washing conditions, the experiment aims to assess the effectiveness of blood detection under different temperature and detergent conditions. [13]

3. RESULT

The fabric washings commenced on 17-01-23, involving absorbent fabrics subjected to ten consecutive washings with and without detergent. Blue color changes, indicative of blood presence, persisted until the tenth washing, irrespective of detergent usage. Subsequently, fabric washings at 50°Celsius initiated on 01-02-2023. After seven washings with detergent, blood stains became undetectable from the eighth washing onwards. However, washings without detergent displayed discernible color changes until the tenth washing, effectively detecting blood stains. Similarly, fabric washings at 100°Celsius, which began on 05-02-2023, demonstrated that after five washings with detergent, blood stains became undetectable from the sixth washing onwards. Conversely, washings without detergent revealed no color changes from the 8th washing. The washings of non-absorbent fabrics, both with and without detergent, commenced on 19-01-2023. After eight consecutive washings, blood stains were undetectable from the ninth washing onward when tested with Benzidine. However, color changes were noted until the tenth washing for fabrics washed without detergent. In another trial starting from 01-02-2023, after six consecutive washings with detergent, blood stains were undetectable from the seventh washing onward at 50 degrees Celsius. Similarly, fabrics washed without detergent did not show color changes from the 9th washing.

Table 1: Observations of absorbent fabric washing done at normal temperature

S. No.	Date	Day	Observations					
			With Detergent			Without Detergent		
			Control Positive	Control Negative	Test	Control Positive	Control Negative	Test
1	17-01-23	Tuesday	+	–	+	+	–	+
2	18-01-23	Wednesday	+	–	+	+	–	+
3	19-01-23	Thursday	+	–	+	+	–	+
4	20-01-23	Friday	+	–	+	+	–	+
5	21-02-23	Saturday	+	–	+	+	–	+
6	22-01-23	Sunday	+	–	+	+	–	+
7	23-01-23	Monday	+	–	+	+	–	+
8	24-01-23	Tuesday	+	–	+	+	–	+
9	25-01-23	Wednesday	+	–	+	+	–	+
10	26-01-23	Thursday	+	–	+ +	+	–	+

For non-absorbent fabrics washed starting on 05-02-2023, after three consecutive washings with detergent, blood stains were undetectable from the fourth washing onward at 100 degrees Celsius. Additionally, fabrics washed without detergent did not exhibit color changes during the 6th washing. These observations highlight the influence of detergent usage on blood stain removal and the varying efficacy of Benzidine in detecting blood stains after multiple washings at different temperatures. Throughout these experiments, the Benzidine test consistently exhibited sensitivity, yielding positive results until the 10th washing, signifying the presence of blood.

Figure 2: Benzidine test on day 1st washed absorbent fabrics with plain water having normal temperature showing positive result

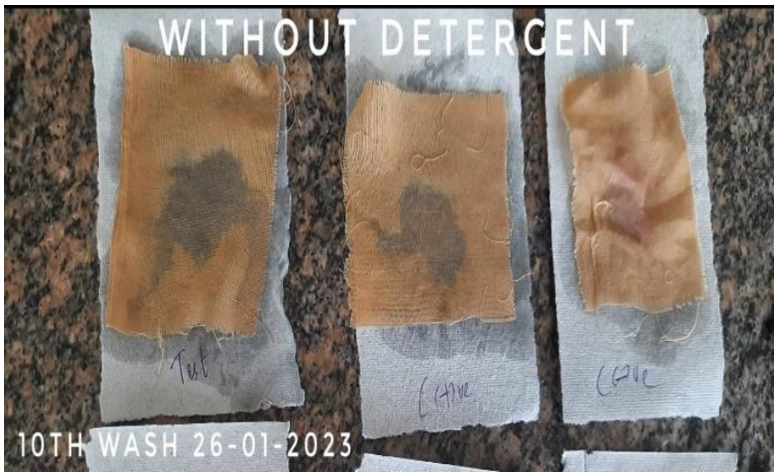

Figure 3: Benzidine test on day 10th washed absorbent fabrics with plain water having normal temperature showing positive result

Figure 4: Benzidine test on day 1st washed absorbent fabrics using detergent at normal temperature showing positive result

Figure 5: Benzidine test on day 1st washed absorbent fabrics using detergent at normal temperature showing positive result

Sl. No.	Date	Day	Observations					
			With Detergent			Without Detergent		
			Control Positive	Control Negative	Test	Control Positive	Control Negative	Test
1	01-02-23	Wednesday	+	–	+	+	–	+
2	02-02-23	Thursday	+	–	+	+	–	+
3	03-02-23	Friday	+	–	+	+	–	+
4	04-02-23	Saturday	+	–	+	+	–	+
5	05-02-23	Sunday	+	–	+	+	–	+
6	06-02-23	Monday	+	–	+	+	–	+
7	07-02-23	Tuesday	+	–	–	+	–	+
8	08-02-23	Wednesday	+	–	–	+	–	+
9	09-02-23	Thursday	+	–	–	+	–	–
10	10-02-23	Friday	+	–	–	+	–	–

Table 2: Observations of absorbent fabric washing done at 50 degrees Celsius

Sl. No	Date	Day	Observations					
			With Detergent			Without Detergent		
			Control Positive	Control Negative	Test	Control Positive	Control Negative	Test
1	05-02-23	Friday	+	–	+	+	–	+
2	06-02-23	Saturday	+	–	+	+	–	+
3	07-02-23	Sunday	+	–	+	+	–	+
4	08-02-23	Monday	+	–	–	+	–	+
5	09-02-23	Wednesday	+	–	–	+	–	+
6	10-02-23	Wednesday	+	–	–	+	–	–
7	11-02-23	Thursday	+	–	–	+	–	–
8	12-02-23	Friday	+	–	–	+	–	–
9	13-02-23	Saturday	+	–	–	+	–	–
10	14-02-23	Sunday	+	–	–	+	–	–

Table 3: Observations of absorbent fabric washing done at 100 degrees Celsius

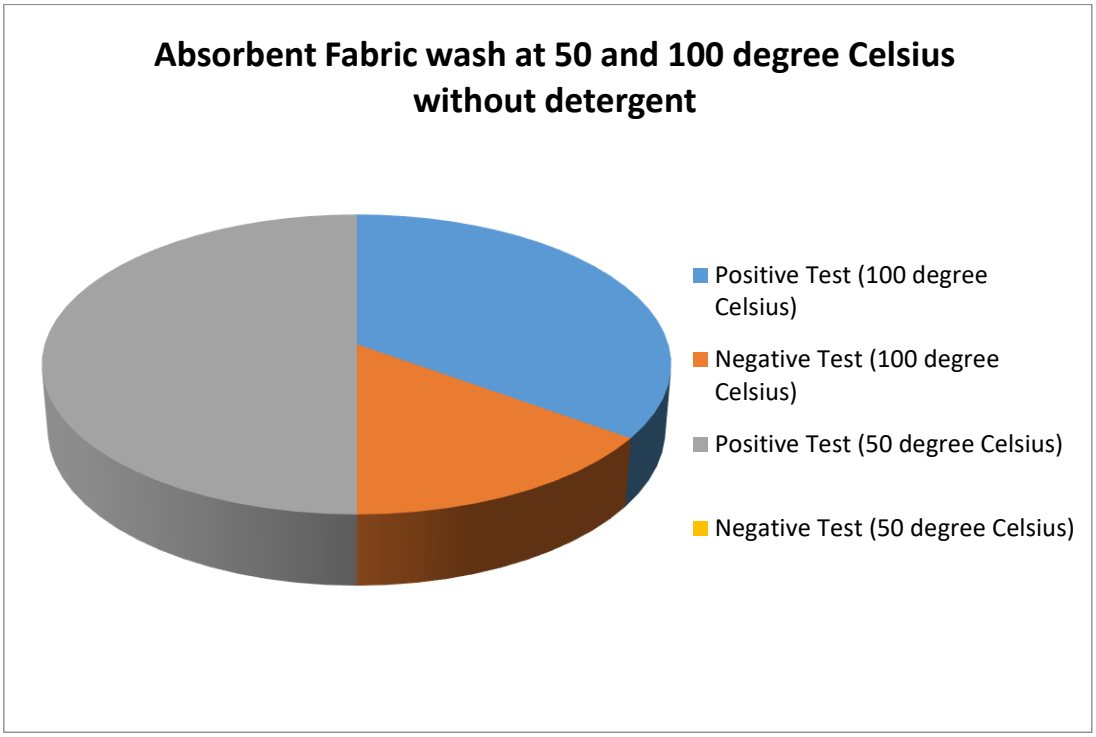

Chart 1: Distribution of tested fabrics based on the presence of blood established using benzidine test on absorbent fabric washed without detergent at 50 and 100 degrees Celsius

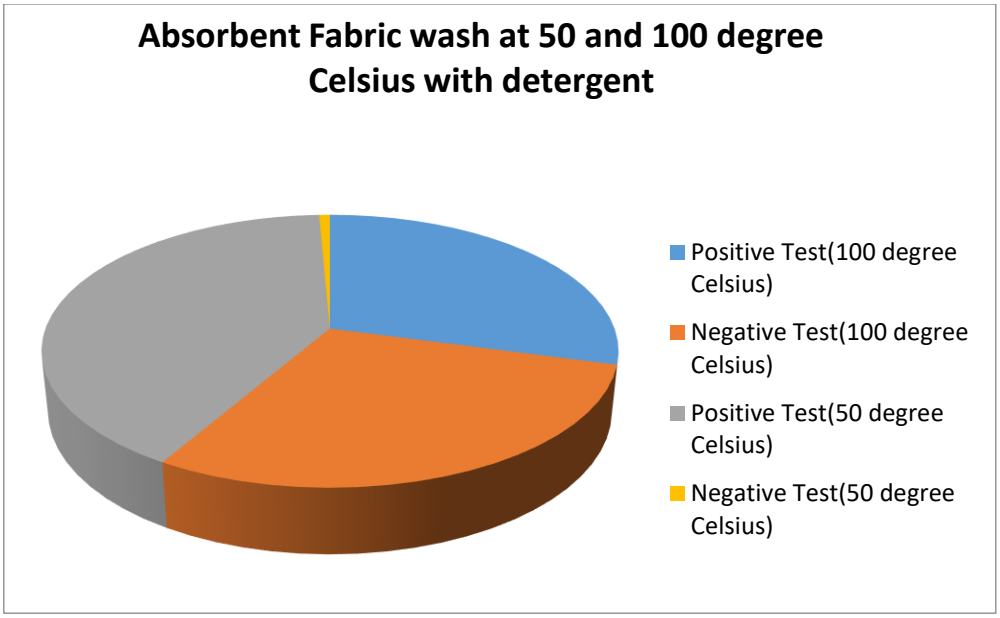

Chart 2: Distribution of tested fabrics based on the presence of blood established using benzidine test on absorbent fabric washed with detergent at 50 and 100 degrees Celsius.

Sl. No	Date	Day	Observations					
			With Detergent			Without Detergent		
			Control Positive	Control Negative	Test	Control Positive	Control Negative	Test
1	01-02-23	Wednesday	+	–	+	+	–	+
2	02-02-23	Thursday	+	–	+	+	–	+
3	03-02-23	Friday	+	–	+	+	–	+
4	04-02-23	Saturday	+	–	+	+	–	+
5	05-02-23	Sunday	+	–	+	+	–	+
6	06-02-23	Monday	+	–	+	+	–	+
7	07-02-23	Tuesday	+	–	+	+	–	+
8	08-02-23	Wednesday	+	–	–	+	–	+
9	09-02-23	Thursday	+	–	–	+	–	+
10	10-02-23	Friday	+	–	–	+	–	+

Table 4: Observations of Non-absorbent fabric washing at normal temperature

Figure 6: Benzidine test on day 1st washed non-absorbent fabrics with plain water having normal temperature showing positive result

Figure 7: Benzidine test on day 9th washed non-absorbent fabrics with plain water having normal temperature showing negative result

Figure 8: Benzidine test on day 1st washed non-absorbent fabrics with plain water having normal temperature showing positive result

Figure 9: Benzidine test on day 10th washed non-absorbent fabrics with plain water having normal temperature showing positive result

Sl. No	Date	Day	Observations					
			With Detergent			Without Detergent		
			Control Positive	Control Negative	Test	Control Positive	Control Negative	Test
1	05-02-23	Friday	+	–	+	+	–	+
2	06-02-23	Saturday	+	–	+	+	–	+
3	07-02-23	Sunday	+	–	+	+	–	+
4	08-02-23	Monday	+	–	+	+	–	+
5	09-02-23	Tuesday	+	–	+	+	–	+
6	10-02-23	Wednesday	+	–	–	+	–	+
7	11-02-23	Thursday	+	–	–	+	–	+
8	12-02-23	Friday	+	–	–	+	–	–
9	13-02-23	Saturday	+	–	–	+	–	–
10	14-02-23	Sunday	+	–	–	+	–	–

Table 5: Observations of Non-absorbent fabric washing done at 50 degrees Celsius

Sl. No	Date	Day	Observations					
			With Detergent			Without Detergent		
			Control Positive	Control Negative	Test	Control Positive	Control Negative	Test
1	05-02-23	Friday	+	–	+	+	–	+
2	06-02-23	Saturday	+	–	+	+	–	+
3	07-02-23	Sunday	+	–	+	+	–	+
4	08-02-23	Monday	+	–	–	+	–	+
5	09-02-23	Tuesday	+	–	–	+	–	+
6	10-02-23	Wednesday	+	–	–	+	–	–
7	11-02-23	Thursday	+	–	–	+	–	–
8	12-02-23	Friday	+	–	–	+	–	–
9	13-02-23	Saturday	+	–	–	+	–	–
10	14-02-23	Sunday	+	–	–	+	–	–

Table 6: Observations of Non-absorbent fabric washing done at 100 degrees Celsius

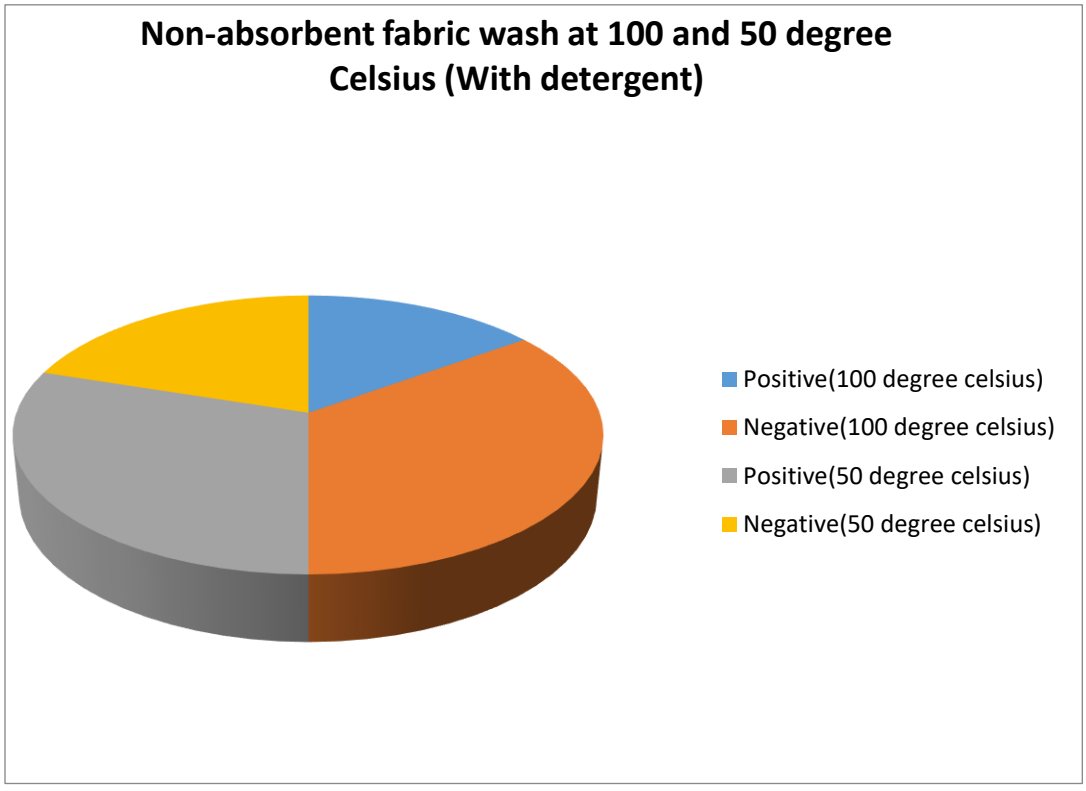

Chart 3: Distribution of tested fabrics based on the presence of blood established using benzidine test on non-absorbent fabric washed with detergent at 50 and 100 degrees Celsius.

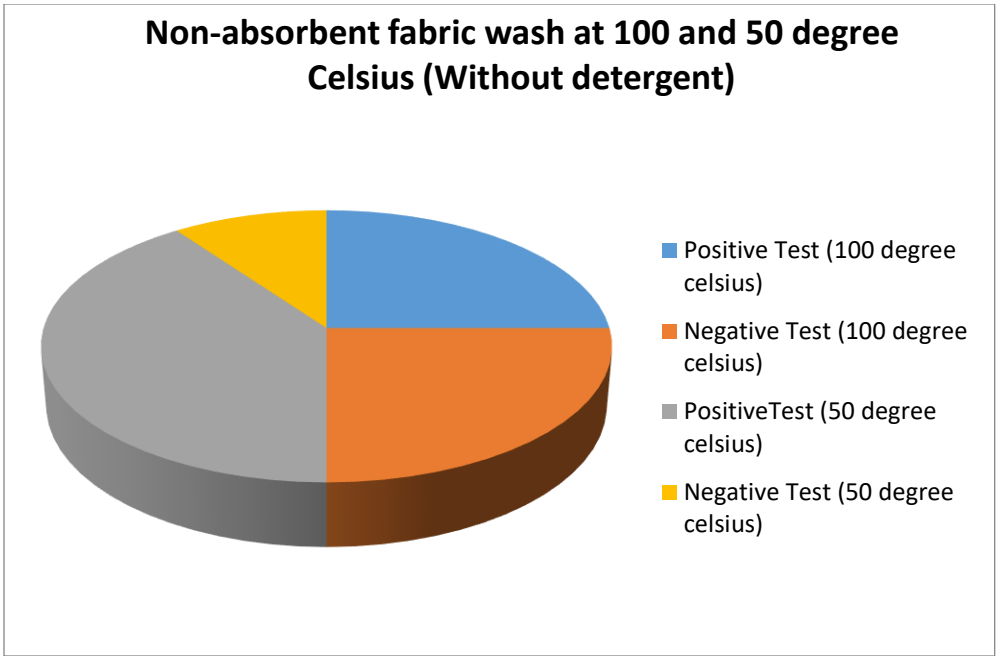

Chart 4: Distribution of tested fabrics based on the presence of blood established using benzidine test on non-absorbent fabric washed without detergent at 50 and 100 degrees Celsius

4. DISCUSSION

The results of the fabric washings and Benzidine tests raise significant considerations regarding the efficacy of Benzidine in detecting blood stains, particularly after multiple washings. Despite initial sensitivity to blood presence, the tests revealed a loss of efficacy over successive washings, indicating potential limitations in its reliability. The effectiveness of benzidine in the detection of bloodstains on washed fabrics is an interesting and practical topic in forensics and criminal investigations. The discussion of this research topic explores the strengths, limitations, and implications of using the benzidine test in this particular application. The washing process can remove or dilute blood stains, making them harder to detect. Several factors can affect the effectiveness of the test, including fabric type, washing conditions (eg. temperature, detergent), and time between dyeing and washing [11] Some studies have reported positive results, suggesting that the test can still detect blood stains on washed fabrics, albeit at a lower rate. This indicates that the benzidine test may have a limited ability to detect partially removed or diluted blood spots after washing. [5] Given the potential limitations of the benzidine test on washed fabrics, forensic scientists and analysts must consider alternative methods or additional methods to improve bloodstain detection. For example, luminol-based tests based on chemiluminescence detection may be more sensitive in detecting residual blood even after washing. DNA tests, such as PCR-based methods, can also provide valuable information to confirm the presence of blood and identify its source. Blood is damaged by temperatures exceeding 50°C, as has been known since Landois' work in 1875. Additionally, some studies have shown that hemolysis happens at 50 °C and that the amount of hemolysis rises linearly with incubation duration. However, a fascinating finding from the study was the color changing of benzidine in the fabrics washed with significantly high temperatures. [21]

5. LIST OF ABBREVIATION

KM – Kastle-TMB…… 2
TMB-Tetra-Methyl Benzidine……2-15
ABO – Antigen A, Antigen B &Antigen H……2
DNA- Deoxyribonuclicacid……15
EDTA- Etheylenediaminetetraacetic acid……9
PCR- Polymerase chain reaction……15
H2O2-Hydrogen Peroxide……5
HIV - Human Immunodeficiency Virus
PPE- Personal protective kit

REFERENCES

1. Rana AK. The Future of Forensic Biology. J Biomed [Internet]. 2018 [cited 2024 Mar 22];3:13-8. Available from: https://doi.org/10.7150/jbm.22760
2. Basu D, Kulkarni R. Overview of blood components and their preparation. Indian J Anaesth [Internet]. 2014 [cited 2024 Mar 22];58(5):529. Available from: https://doi.org/10.4103/0019-5049.144647
3. Forensic Science Reform [Internet]. [place unknown]: Elsevier; 2017. Presumptive and Confirmatory Blood Testing; [cited 2024 Mar 22]; p. 239-69. Available from: https://doi.org/10.1016/b978-0-12-802719-6.00008-x
4. Butler J, Chaseling J, Wright K. A Comparison of Four Presumptive Tests for the Detection of Blood on Dark Materials. J Forensic Sci [Internet]. 2019 May 31 [cited 2024 Mar 22];64(6):1838-43. Available from: https://doi.org/10.1111/1556-4029.14091

5. Garner DD, Cano KM, Peimer RS, Yeshion TE. An Evaluation of Tetramethylbenzidine as a Presumptive Test for Blood. J Forensic Sci [Internet]. 1976 Oct 1 [cited 2024 Mar 22];21(4):10566J. Available from: https://doi.org/10.1520/jfs10566j
6. Gupta S, Garg A, Babu S, Yadav DS. Comparative study of presumptive and confirmatory tests for detection of blood on serial dilutions and washed stains. Int J Health Res Medico Leg Pract [Internet]. 2021 Jun 15 [cited 2024 Mar 22];7(1). Available from: https://doi.org/10.31741/ijhrmlp.v7.i1.2021.10
7. Gomes C, López-Matayoshi C, Palomo-Díez S, López-Parra AM, Cuesta-Alvaro P, Baeza-Richer C, Gibaja JF, Arroyo-Pardo E. Presumptive tests: A substitute for Benzidine in blood samples recognition. Forensic Sci Int [Internet]. 2017 Dec [cited 2024 Mar 22];6:e546-e548. Available from: https://doi.org/10.1016/j.fsigss.2017.09.213
8. Spector, J., & Von Gemmingen, D. (1971). The Effect of Washing on the Detection of Blood and Seminal Stains. *Journal of the Canadian Society of Forensic Science*, *4*(1), 3– https://doi.org/10.1080/00085030.1971.10757279
9. De Vittori E, Barni F, Lewis SW, Antonini G, Rapone C, Berti A. Forensic application of a rapid one-step tetramethylbenzidine-based test for the presumptive trace detection of bloodstains at the crime scene and in the laboratory. Forensic Chem [Internet]. 2016 Nov [cited 2024 Mar 22];2:63-74. Available from: https://doi.org/10.1016/j.forc.2016.10.002
10. Cox M. A Study of the Sensitivity and Specificity of Four Presumptive Tests for Blood. J Forensic Sci [Internet]. 1991 Sep 1 [cited 2024 Mar 22];36(5):13170J. Available from: https://doi.org/10.1520/jfs13170j Stojanović, I. (2019). DETECTION OF BLOODSTAINS ON COTTON FABRIC AFTER WASHING. *Acta Medica Medianae*, *58*(1), 24–27. https://doi.org/10.5633/amm.2019.0104
11. Hofmann M, Adamec J, Anslinger K, Bayer B, Graw M, Peschel O, Schulz MM. Detectability of bloodstains after machine washing. Int J Leg Med [Internet]. 2018 Jul 21 [cited 2024 Mar 22];133(1):3-16. Available from: https://doi.org/10.1007/s00414-018-1897-2
12. Verma H. Benzidine color-crystal test; Modified test to confirm the presence of blood stain. Forensic Sci Int [Internet]. 2022 Apr [cited 2024 Mar 22]:111313. Available from: https://doi.org/10.1016/j.forsciint.2022.111313
13. Rao I A. Identification of Blood Stains on Different Fabrics after Washing with Routinely Used Detergents in India. Int J Forensic Sci [Internet]. 2016 [cited 2024 Mar 22];1(1). Available from: https://doi.org/10.23880/ijfsc-16000102
14. Smith MA, Dahlen NR, Bruemmer A, Davis S, Heishman C. Clinical Practice Guideline Surgical Site Infection Prevention. Orthop Nurs [Internet]. 2013 [cited 2024 Mar 22];32(5):242-8. Available from: https://doi.org/10.1097/nor.0b013e3182a39c6b
15. Nordstrom JM, Reynolds KA, Gerba CP. Comparison of bacteria on new, disposable, laundered, and unlaundered hospital scrubs. Am J Infect Control [Internet]. 2012 Aug [cited 2024 Mar 22];40(6):539-43. Available from: https://doi.org/10.1016/j.ajic.2011.07.015
16. Blaser MJ, Smith PF, Cody HJ, Wang WL, LaForce FM. Killing of Fabric-Associated Bacteria in Hospital Laundry by Low-Temperature Washing. J Infect Dis [Internet]. 1984 Jan 1 [cited 2024 Mar 22];149(1):48-57. Available from: https://doi.org/10.1093/infdis/149.1.48
17. Fijan S, Šostar-Turk S. Antimicrobial activity of selected disinfectants used in a low temperature laundering procedure for textiles. Fibres Amp Text East Eur. [2010] . Available from: http://fibtex.lodz.pl/pliki/Fibtex_(no65e4krqz116rnj).pdf].
18. Altenbaher B, Šostar Turk S, Fijan S. Ecological parameters and disinfection effect of low-temperature laundering in hospitals in Slovenia. J Clean Prod [Internet]. 2011 Jan [cited 2024 Mar 22];19(2-3):253-8. Available from: https://doi.org/10.1016/j.jclepro.2010.10.002
19. Banik RM, Prakash M. Laundry detergent compatibility of the alkaline protease from Bacillus cereus. Microbiol Res [Internet]. 2004 Jun [cited 2024 Mar 22];159(2):135-40. Available from: https://doi.org/10.1016/j.micres.2004.01.002
20. De Almeida JP, Glesse N, Bonorino C. Effect of presumptive tests reagents on human blood confirmatory tests and DNA analysis using real time polymerase chain reaction. Forensic Science International. 2011;206:58-61.