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Efficacy of Tetramethylbenzidine in Forensics Investigation for the Detection of Blood on Fabrics of Different Texture

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ABSTRACTS : Blood stain analysis is an important aspect of crime investigation. The presence of blood on seized exhibits can relate a suspect with the crime and help in reconstructing the crime scene. Clothes of different texture seized from victims, accused, deceased or crime scene are important for forensic examination and thus for further investigation. It is often found that attempts are taken to remove the blood stain by washing with detergents and application of different chemicals. Benzidine, Phenolphthalein, Tetramethylbenzidine, Leucomalachite green and orthotolidine solution are widely used as test reagents detecting the presence of blood in forensic examination.

In the present paper 10 different types of blood soaked natural and manmade fabrics washed with detergent and tap water. Blood stained fabrics were washed with frequently used commercially available detergent. The purpose of this study was to investigate the efficacy of Tetramethylbenzidine and phenolphthalein separately in detecting blood stains on clothes.

KEYWORDS : Tetramethylbenzidine, Fabrics, Phenolphthalein, Forensics, Blood stain.

I. INTRODUCTION

The most common and important biological stain that requires identification and characterization of crime is blood. Investigation and examination of biological evidences present at the crime scene plays an important role in linking the criminal with the crime¹. The most important task for the expert or scientist is to examine seized materials from crime scene and trace the minute trace evidence left by the criminal. After that the collected material is examined by different scientific methods to produce the right testimony of both the victim and the suspect (2).

There are many reliable methods for the detection and identification of blood stains. For many years, forensic science laboratories relied heavily upon the benzidine test to screen for blood. Because of the carcinogenic effects of benzidine, other suitable replacements have been sought by laboratories. These include phenolphthalein and Tetramethylbenzidine which change color when they come in contact with peroxides or hemoglobin in the blood (3). Most of the biological evidence materials remain stuck to the clothes like adhesive in the fabrics. Besides every possibility of removing the blood stains from crime scene, criminals try to remove blood stains by washing the surface, floor, tiles and other objects like garments (4,5). Due to this, blood cannot be seen through naked eye after washing (6). For the detection of minute amounts of blood traces and assumed bloodstains, the presumptive Tetramethylbenzidine (TMB) test and phenolphthalein test are widely used in forensic examination. (6,7).

An evaluation of the tetramethylbenzidine test as presumptive test for blood was reported by Garner, Cano, Peimer and Yeshion in 1976 (8). These authors observed no significant difference between the benzidine and tetramethylbenzidine tests in sensitivity or specificity (8). The principle of Tetramethylbenzidine (TMB) test is that the heme compound in blood reacts with TMB in the presence of oxidizing agent leading to oxidation of TMB to form a blue-green colored



product.Reduced Phenolphthalein test is also known as Kastle-Meyer test (04). Phenolphthalein reaction takes place in an alkaline solution to yield an immense pink color on reacting with the heme portion of blood. The heme group of hemoglobin exhibits a “peroxidase like” activity, which may catalyze the breakdown of hydrogen peroxide (09).

II. MATERIALS AND METHOD

This study was carried out in our State Forensic Science Laboratory, Raipur (C.G.). Sample blood was obtained from nearby blood bank. Sample blood was applied on two similar set of ten selected natural and manmade clean clothes, dried at room temperature for 24 hours separately. Next day one set of clothes was dipped in a beaker containing tap water and the in a beaker containing detergent water. Both were kept for 05 minutes. Detergent washed clothes were rinsed in tap water individually, and all clothes were dried separately for 24 hours at room temperature. From 3rd day onward control clothes, blood soaked- tap water washed and blood soaked-detergent washed clothes were examined with Tetramethylbenzidine solution and phenolphthalein (Karl Mayer test) separately (one washing per day) for all ten types of clothes up to ten consecutive days.

Tetramethylbenzidine gave positive results with appearance of bluish-green color and phenolphthalein gave positive results with the appearance of pink color.

III. RESULT AND DISCUSSION

Table 1: Reaction of blood stains with Tetramethylbenzidine

Type of Cloths	Tap water wash (In days)										Detergent wash (in days)									
	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Hosiery	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Jeans	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
Cotton	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Woolen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Rayon	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-	-	-	-	-
Silk	+	+	+	+	+	+	+	+	-	-	+	+	+	+	-	-	-	-	-	-
Polycotton	+	+	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Jute	+	+	+	+	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-
Chiffon	+	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Polyester	+	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-

+ : Blood stains found positive
 - : Blood stains found Negative

Table 2: Reaction of blood stains with Phenolphthalein

TYPE OF CLOTHS	Tap water WASH (IN DAYS)										DETERGENT WASH (IN DAYS)									
	1	2 nd	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Hosiery	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Jeans	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
Cotton	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Woolen	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	-	-	-	-
Rayon	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
Silk	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polycotton	+	+	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Jute	+	+	+	+	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-
Chiffon	+	+	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Polyster	+	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-

+ : Blood stains found positive



- : Blood stains found Negative

Table 3:Efficacy of Tetramethylbenzidine and Phenolphthaleinon Tap water washed clothes

S.No.	Fabrics	Tetramethylbenzidine(+ve)	Phenolphthalein(+ve)	Efficacy (blood +ve)
1	Hosiery	10	10	Same
2	Jeans	10	10	Same
3	Cotton	10	10	Same
4	Woolen	10	7	3 more (Benz)
5	Rayon	9	10	1more (phenol.)
6	Silk	8	10	2more (phenol.)
7	Polycotton	6	6	Same
8	Jute	5	5	Same
9	Chiffon	3	4	1more (phenol.)
10	Polyester	2	2	Same

Table 3 shows that Tetramethylbenzidine and phenolphthalein both gave positive result up to 10 washes for tap water hosiery, jeans and cotton, up to 06 washes for polycotton, up to 05 washes for jute and up to 02 washes for polyesterblood-stained clothes.

Tetramethylbenzidine gave 03 more positive results for woolen clothes,phenolphthalein gave 02 more positive result for silk and 01 more positive result for rayon and chiffon blood-stained clothes washed with tap water.

Table.4: Efficacy of Tetramethylbenzidine and phenolphthalein on detergent water washed

S.No.	Fabrics	Tetramethylbenzidine(+ve)	Phenolphthalein(+ve)	Efficacy(blood+ve)
1	Hosiery	10	09	1 more (Benz.)
2	Jeans	6	7	1 more(phen.)
3	Cotton	10	10	same
4	Woolen	10	6	4 more (Benz.)
5	Rayon	3	3	same
6	Silk	4	10	6 more(phen.)
7	Polycotton	1	1	same
8	Jute	2	2	same
9	Chiffon	1	1	same
10	Polyster	2	2	same

Table4 shows that Tetramethylbenzidine and phenolphthalein both gavepositive result up to 10 washings for detergent washedcotton, up to 03 washings for rayon, up to 02 washing for jute andpolyesterup to 01 washing for polycotton and chiffonblood-stained clothes.

Tetramethylbenzidine gave 01 more positive result for hosiery clothes, 04 more positive results for woolen clothes.Phenolphthalein gave 06 more positive results for silk and 01 more positive result for jeans blood-stained clothes.

Table 5:Efficacy of Tetramethylbenzidine for washed with tap water and with detergent

S.No.	Fabrics	Tap water washed(+ve)	Detergentwashed (+ve)	Efficacy(blood+ve)
1	Hosiery	10	10	same
2	Jeans	10	6	4 more (Benz.)
3	Cotton	10	10	same
4	Woolen	10	10	same



5	Rayon	9	3	6more (Benz)
6	Silk	8	4	4more (Benz)
7	Polycotton	6	1	5more (Benz)
8	Jute	5	2	3more (Benz)
9	Chiffon	3	1	2more (Benz)
10	Polyester	2	2	same

Table 5 shows that in tap water washed blood-stained hosiery, jeans, cotton and woolen clothes, Tetramethylbenzidine gave positive results up to 10 washes, Rayon up to 09 washes, silk up to 08 washes, polycotton up to 06 washes, jute up to 05 washes chiffon up to 3 washes and up to 02 washes for polyester blood-stained clothes. In detergent washed hosiery, cotton and woolen clothes, Tetramethylbenzidine gave positive results up to 10 washes, jeans up to 06 washes, silk up to 04 washes, Rayon up to 03 washes, polyester and jute up to 02 washes, polycotton and Chiffon up to 01 wash for blood stained clothes.

Table 6: Efficacy of phenolphthalein for fabrics washed with tap water and with detergent

S.No.	Fabrics	Tap water washed (+ve)	detergent washed (+ve)	Efficacy (blood+ve)
1	Hosiery	10	09	1more (Benz)
2	Jeans	10	7	3more (Benz)
3	Cotton	10	10	same
4	Woolen	7	6	1more (Benz)
5	Rayon	10	3	7more (Benz)
6	Silk	10	10	same
7	Polycotton	6	1	5more (Benz)
8	Jute	5	2	3more (Benz)
9	Chiffon	4	1	3more (Benz)
10	Polyester	2	2	same

Table 6 shows phenolphthalein gave positive results up to 10 washes for cotton and silk and Polyester up to 02 washes for both tap water washed and detergent washed blood-stained clothes, up to 07 washes for woolen cloth, 06 for polycotton, 05 for jute, 04 for chiffon and 02 for polyester in case of tap water washed blood-stained clothes, whereas in case of detergent washed jeans, phenolphthalein gave positive result up to 07 washes, woolen clothes up to 06 washes, rayon up to 03 washes, jute, and polyester up to 02 washes, polycotton and chiffon up to 01 wash.

Tetramethylbenzidine and phenolphthalein gave higher frequency of positive results in all 10 selected blood-stained clothes for tap water washing than detergent washing. Phenolphthalein was found to be more sensitive for tap water washed, silk and chiffon, detergent washed jeans and silk clothes. Hosiery, Jeans, cotton, woolen and silk clothes showed maximum retention of blood even after washing with detergent whereas chiffon, polycotton and polyester had the minimum ability to hold stain.

IV. CONCLUSION

Analysis of blood stain recovered from crime scene is an extremely specialized area, which can often prove to be the most valuable source of information, when searching for clues and details of the event (12). In our study Tetramethylbenzidine test and phenolphthalein test were used to detect the presence of blood on clothes washed with tap water and detergent washed clothes.

Tetramethylbenzidine test yielded positive results for hosiery, cotton and woolen clothes for both tap water wash and detergent wash whereas phenolphthalein test yielded positive results only for cotton and silk fabrics. This implicates that Tetramethylbenzidine test is more sensitive than the phenolphthalein test for presumptive test of blood. Similar result was noted by Cox M (1990). Further confirmatory analysis is time consuming so that the preliminary examination is useful to test all types of stains which could potentially be blood. The basic information revealed after performing the study is that there is a probability of finding blood stains by TMB & KM test on different fabrics even after washing them with detergent. The study paves the way to a raw approach to elucidate the kind of textile seized as



exhibit that would yield more relevant result in laboratory examination for the presence of blood and for further examination to determine the blood group.

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