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Effect of Fabric Washing on the Presumptive Identification of Bloodstains

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ABSTRACT: The purpose of this study was to investigate the retention of blood stains on twelve different types of fabrics after washing at various drying times. The findings of this study, supported by chi-square analysis, indicate that the retention of bloodstains on washed fabrics depends upon the particular fiber composition of the fabric, the specific blood screening test used, and whether or not a detergent was used in the wash. The results of this research did not reveal a significant effect of the drying time on the retention of bloodstains, as tested during the 48-h limit of this experiment. The author concludes that the forensic serologist should consider the factors investigated in this study before rendering an opinion on the retention of bloodstains on washed garments.

KEYWORDS: forensic science, criminalistics, blood, fabrics, forensic serologist, bloodstains, washing, presumptive identification, phenolphthalein, *o*-tolidine

Would washing have removed the blood from the garment? This question is frequently asked the forensic serologist, either by investigating officers or by court counselors. Forensic serologists are aware of a number of potential variables that could affect the identification of blood on garments that have been washed. Some of these possible variables include the age of the stain, the type of fabric, whether or not a detergent was used in the washing, environmental conditions, the temperature of wash water, and the testing procedure utilized.

A search of the literature revealed little on this topic. Jain and Singh [*1*] investigated the detection and origin of bloodstains on various types of cloth immersed in water up to a period of 20 days. In that study, positive benzidine and phenolphthalein tests were made within the 20-day testing period.

Spector and Von Gemmingen [*2*] studied the effect of washing on the detection of blood using the benzidine test. In their study, twelve different washing procedures were used on two types of fabric, cellulose acetate and cotton. Their findings indicated that cotton had a strong affinity for bloodstain, while acetate was less likely to retain blood after being subjected to washing. All bloodstains in that study were allowed to air dry for 24 h.

The specific purpose of the present study was to determine the following relationships:

(*a*) between the fabric type and the retention of bloodstains after drying for specific time periods and washing with a chlorine-containing detergent (Clorox),

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(*b*) between the bloodstain drying time and the retention of blood after washing with a detergent, and

(*c*) between the sensitivity of the orthotolidine and the phenolphthalein tests, as used for screening the stained fabrics.

Materials and Methods

Table 1 lists the twelve different fabrics used in this study and their fiber composition and color. Fresh human blood was used to stain the various fabrics. Each stain was made using 1 mL of blood. The blood stains ranged from 4 to 8 cm in diameter. The size varied with the type and thickness of the fabric. The stains were air dried in an atmosphere with a temperature range of 80°F to 95°F (26.6°C to 35°C).

One bloodstain from each of the twelve fabrics was machine-washed every 3 h over a period of 48 h. The fabrics were washed in a gyrator type of washing machine set on "normal wash." Cold water, as well as a chlorine-containing detergent with color-safe bleach, was used in each washing cycle to enhance the solubility of the bloodstains.

The fabrics were washed, then allowed to air dry before testing with phenolphthalein and orthotolidine reagents for the possible presence of remaining blood. The area of each original bloodstain was marked by making cuts on the periphery of each stain to aid in locating the stained area subsequent to washing.

The phenolphthalein and the orthotolidine tests were selected as the screening tests. These reagents were freshly prepared and used in accordance with the standard methods [*3,4*].

An unstained piece of each fabric was subjected to the same washing conditions as the stained fabrics. Small pieces of the bloodstained area were cut from each fabric and tested with the phenolphthalein and *o*-tolidine reagents. An unstained control was tested with each fabric. The results of the screening tests are recorded in Table 2.

Four hypotheses were tested in this study:

1. There is an effect of the fabric type on the retention of washed bloodstain, based on the *o*-tolidine screening test.
2. There is an effect of the fabric type on the retention of washed bloodstain, based on the phenolphthalein screening test.

TABLE 1—*Fabrics used in testing.*

Fabric No.	Fiber Composition	Article	Color
1	100% acetate	standard cloth	black
2	100% acrylic	sweater	medium blue
3	100% cotton	standard cloth	white
4	100% nylon	nightgown	pale green
5	100% polyester	standard cloth	bright green
6	100% rayon	standard cloth	navy blue
7	100% wool	military blanket	green
8	84% polyester 16% nylon	shirt	white/gold
9	80% polyester 20% cotton	shirt	tan
10	65% polyester 35% cotton	shirt	tan/brown
11	50% polyester 50% cotton	standard cloth	white
12	50% polyester 50% rayon	standard cloth	pale green

TABLE 2—*Blood screening results of twelve washed fabrics.*[a]

Fabric No.	Unstained Washed Control		Drying Time of Bloodstain Before Washing and Screening Results After Washing																													
			3 h		6 h		9 h		12 h		15 h		18 h		21 h		24 h		27 h		30 h		33 h		36 h		39 h		42 h		45 h		48 h	
	P	OT	P	OT	P	OT	P	OT	P	OT	P	OT	P	OT	P	OT	P	OT	P	OT	P	OT	P	OT	P	OT	P	OT	P	OT	P	OT
1	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
2	−	−	−	−	−	−	−	−	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+
3	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
5	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
6	−	−	−	−	−	−	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+
7	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	−	−	−	−	−	−	−	−	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+
9	−	−	−	−	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+
10	−	−	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	−	−	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+

[a]Key to abbreviations:
P = phenolphthalein test.
OT = *o*-tolidine test.
− = negative result.
+ = positive result.

3. There is an effect of the bloodstain drying time on the retention of washed bloodstain, based on the *o*-tolidine and phenolphthalein screening tests.
4. There is a difference between the sensitivity of the *o*-tolidine test and that of the phenolphthalein test, as utilized in this study.

The chi-square test for independence was used in this study to test the hypotheses. In order to have a meaningful consideration, the probability level of 0.01 was chosen as the criterion for significance.

Results

Table 3 presents the observed frequencies for the twelve fabrics tested and the results of the *o*-tolidine and phenolphthalein tests. Of the twelve fabrics tested, three failed to yield a positive presumptive test for blood during the 48-h testing period. These fabrics were all 100% synthetic fibers, namely, acetate, nylon, and polyester. Two of the tested fabrics, Nos. 3 and 11, gave positive results for both the *o*-tolidine and phenolphthalein on all washed stains, starting with the first 3-h drying time through the 48-h period. Both of the fabrics contained cotton: one was composed of 100% cotton and the other of 50% cotton. It was noted that the fabric composed of 50% polyester and 50% rayon, Fabric 12, yielded a positive *o*-tolidine reaction throughout the 48-h testing period. This same fabric failed to give a positive phenolphthalein test at any of the testing stages. Fabric 10, which contained 35% cotton, gave positive *o*-tolidine and positive phenolphthalein results, starting with the 9-h period of drying. Fabric 9, 20% cotton, yielded positive *o*-tolidine results, starting with the 6-h drying period. This fabric gave a negative phenolphthalein result during the entire 48-h testing limit. See Table 2 for the test results.

All of the washed unstained control fabrics gave negative *o*-tolidine and negative phenolphthalein results. It was noted that some of the fabrics, Nos. 4, 8, 9, and 12, gave a light green to blue-green color after a period of 1 to 2 min. These color changes may be interpreted as false positives if not conducted along with a known bloodstain. A positive *o*-tolidine test is indicated by an almost instant blue color reaction when 3% hydrogen peroxide is added to the specimen and *o*-tolidine reagent.

The fabrics tested with phenolphthalein did not present any questionable color reactions. The phenolphthalein test will produce a pink to rose-colored reaction in the presence of blood when one to two drops of 3% hydrogen peroxide is added.

TABLE 3—*Observed frequencies for fabrics and test results using* *o*-tolidine and phenolphthalein.

	o-Tolidine		Phenolphthalein	
Fabric	Positive	Negative	Positive	Negative
1	0	16	0	16
2	13	3	0	16
3	16	0	16	0
4	0	16	0	16
5	0	16	0	16
6	14	2	0	16
7	13	3	13	3
8	13	3	0	16
9	15	1	0	16
10	16	0	14	2
11	16	0	16	0
12	16	0	0	16

The chi-square values testing the relationship between both the *o*-tolidine and phenolphthalein tests and the various types of fabrics were statistically significant at the 0.01 level. The contingency coefficient for the two screening tests indicated a strong relationship between the two variables, the test procedures, and the blood testing results. The contingency coefficient was 0.6878 for the phenolphthalein test and 0.6565 for the *o*-tolidine test. Chi-square statistics for these data are included in Tables 4 and 5.

Examination of Table 2 reveals the consistency of the test results, from the 3-h period through the 48-h period, for most of the fabrics tested. Fabrics 1, 3, 4, 5, 10, 11, and 12 gave consistent results in all testing periods.

The chi-square for independence test indicated that there was not a significant relationship between the drying periods and the blood testing results. The obtained significance level of 0.3885 is far greater than the hypothesized level of 0.01. The chi-square results are presented in Table 6.

The *o*-tolidine test yielded 132 positive results to only 59 positives for the phenolphthalein test when used on the same washed bloodstain. The phenolphthalein test had 133 negative results to 60 for the *o*-tolidine. There were 192 frequencies or possible results.

In an attempt to evaluate or compare the sensitivity of the *o*-tolidine test to that of the phenolphthalein procedure, a chi-square for independence was utilized. The significance level for the variables was identified as being 0.0001. This level is far below the 0.01 level being tested. See Table 7 for the chi-square results.

TABLE 4—*Chi-square test for independence for the phenolphthalein testing of twelve fabrics.*

Number of observations	192
Chi-square	172.3278
Degrees of freedom	11
Significance level	0.0001
Contingency coefficient	0.6878

TABLE 5—*Chi-square test for independence for the o-tolidine testing of twelve fabrics.*

Number of observations	192
Chi-square	145.4546
Degrees of freedom	11
Significance	0.0001
Contingency coefficient	0.6565

TABLE 6—*Chi-square for independence for stain drying periods and test results.*

Number of observations	192
Chi-square	15.9030
Degrees of freedom	15
Significance level	0.3885
Contingency coefficient	0.2766

TABLE 7—*Chi-square test for independence for o-tolidine and phenolphthalein testing procedures and results.*

	Observed Frequencies	
	Positive	Negative
Phenolphthalein	59	133
o-Tolidine	132	60
Number of observations		384
Chi-square		55.5119
Yates' correction		54.0015
Degrees of freedom		1
Significance		0.0001
Contingency coefficient		0.3554

This study revealed several interesting findings which are supported by statistical data:

1. The first hypothesis, which predicted that there is an effect of the fabric type on the retention of washed bloodstain based on the *o*-tolidine test, was accepted based on the obtained significance level of 0.0001. The same level of significance was also obtained for the testing of washed bloodstains with phenolphthalein in the second hypothesis.
2. The findings in this study did not support the third hypothesis. It was not established that there was a significant effect of the bloodstain drying time on the retention of washed bloodstain, as tested within the limits of this project.
3. The fourth hypothesis, which predicted a difference in sensitivity between *o*-tolidine and phenolphthalein tests, was also accepted based on the obtained significance level of 0.0001.

Discussion

The findings in this study clearly indicate a relationship between the type of fabric and the retention of bloodstains. This was illustrated by the absence of blood in Fabrics No. 1 (100% acetate), 4 (100% nylon), and 5 (100% polyester) during the limit of the study and the detection of blood in the cotton fabrics (Nos. 3, 9, 10, and 11).

This study failed to reveal a significant relationship between the blood drying time before washing and the retention of blood by the fabrics. It most instances, blood was either not detected throughout the whole 48-h period or it was detected during most of the study by either the *o*-tolidine, the phenolphthalein test, or both tests.

It was also obvious in this study that there was a significant difference in sensitivity between the *o*-tolidine and phenolphthalein tests. Of the fabrics tested, only Fabrics No. 3 (100% cotton), 7 (100% wool), 10 (65% polyester/35% cotton), and 11 (50% polyester/50% cotton) gave positive results with both *o*-tolidine and phenolphthalein. It was noted that cotton was present in each of these fabrics, with the exception of the 100% wool sample.

The subject study was conducted after the stained fabrics had been washed with a chlorine-containing detergent. In an effort to evaluate the importance of using a detergent in the wash water, bloodstains on each of the twelve fabrics were allowed to dry 24 h and were washed without the chlorine-containing detergent. All twelve stained fabrics gave positive results for both *o*-tolidine and phenolphthalein. After this same drying period, when washed with the detergent, only Fabrics No. 3 (100% cotton), 7 (100%

wool), 10 (65% polyester/35% cotton), and 12 (50% polyester/50% cotton) gave positive *o*-tolidine and phenolphthalein results.

Conclusions

The following conclusions were drawn from this study:

1. The retention of bloodstains on washed fabrics depends upon the fiber composition.
2. The drying time of the bloodstains, within the 48-h limit of this study, is not significant to the retention of bloodstains when the washing procedure utilized in this study is followed.
3. The results of the blood screening test depend upon the particular screening procedure used. The *o*-tolidine test was found to be more sensitive than the phenolphthalein test in this study.
4. Fabrics washed with a chlorine-containing detergent are less likely to retain bloodstains than those washed without the detergent. Cold water was also used in this study to enhance the removal of the bloodstains.

The author concludes that the forensic serologist should consider the factors investigated in this study before rendering an opinion regarding the presumptive identification of blood on clothes that have been washed.

References

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