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Extraction of DNA from Bloodstained Fabrics Exposed to Different Environmental Conditions

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Bloodstains are one of the most common pieces of biological evidence found at the scene of a violent crime. Bloodstains found at a crime scene usually occur on items of clothing. Thus, blood on fabric needs to be understood. This research was conducted to identify the types of fabrics that retain a higher concentration of DNA and the persistence of this DNA when exposed to different environmental conditions such as sunlight, high humidity and heavy rain. Three different locations were used in this study to mimic real crime scenes, namely a jungle, an abandoned building and a construction site. Ten different types of fabrics used for this study were satin, chiffon, linen, polyester, cotton, crepe, argenti, valentro, lycra and georgette. The fabric samples were stained with fresh human blood and exposed to different environmental conditions for seven days. Samples were collected every 24 hours. DNA extraction was done using a NEXprep^{TM} NexK-3100 Genomic Mini Kit. The concentration of DNA was measured using BioPhotometer Plus by Eppendorf. The type of fabric that retained the highest concentration of DNA when exposed under three different environmental conditions was crepe. The concentration of DNA retained by the fabric samples in an indoor abandoned building was higher compared to the concentration of DNA retained by the fabric samples in an outdoor jungle area and outdoor construction site. The amount of DNA recovered from these fabrics varied according to the different environmental conditions. The possibility for DNA contamination increased with time as the bloodstained fabrics were exposed to the uncontrolled environment. Therefore, the biological materials, substrates and effects of environmental exposure need to be properly understood and studied by forensic scientists so that the results of such analyses may be correctly determined and interpreted.

Key words: Fabrics; DNA; abandoned building; jungle; construction site; environmental exposure

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DNA analysis in forensic science deals with evidence recovered from a crime scene and can be used to link suspects to crime scenes [1]. A single drop of blood can link a suspect to a victim, a crime scene and a weapon they used. Blood and bloodstains are crucial for solving violent crimes, sexual assaults, vehicular accident cases and many more [2]. DNA as biological evidence in the forensic science field has become increasingly important in criminal investigations. The challenge is when evidence at the crime scene is exposed to extreme weather that can cause contamination, such as strong UV radiation, rain, flooding and many more. Solar ultraviolet radiation is one of the most critical environmental DNA-damaging agents [3]. When DNA is exposed to UV radiation, it will cause chemical changes which can alter the shape of DNA and also change the DNA code [4]. Contamination of DNA and proteins are highly dependent on the presence of water because it can enhance microbial growth that leads to the rapid decay of DNA [5]. Contamination is a serious problem that can destroy evidence and end up jeopardizing a criminal case. It is impossible to completely prevent contamination [6].

Fabric is produced by weaving and knitting textile fibres into cloth for human daily usage. Two types of fibres that make up fabric are natural fibres and synthetic fibres (manufactured fibres). Natural fibres are derived entirely or wholly from animal or plant sources, while synthetic fibres are derived from either natural or synthetic polymers [7].

Blood is often evidence of a violent crime and may be present on the perpetrator's clothing. In certain circumstances, blood can indicate that the clothing was present during the bloodletting event and identify a potential victim or perpetrator, or provide additional evidence of the crime [8]. This study attempts to investigate the effects of different environmental conditions on biological evidence such as blood-stained fabrics, as well as to identify the fabrics that

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retained a higher concentration of DNA, and the persistence of DNA on these fabrics when exposed to different environmental conditions.

MATERIALS AND METHODS

Sample Preparation

Ten types of pure fabrics, satin, chiffon, linen, polyester, cotton, crepe, argenti, valentro, lycra and georgette, were purchased from the same store. The selected fabrics are commonly used in Malaysia and the fabrics were categorised into natural fabrics (cotton, satin, chiffon, linen) and synthetic fabrics (crepe, argenti, valentro, lycra, lycra, georgette, polyester). The fabrics were cut into square pieces of about 14cm x 14cm. Each fabric sample was prepared in three replicates for three different places: an indoor abandoned building, an outdoor construction site and an outdoor jungle area. A pooled blood source was obtained for this study and about 3.5ml was poured on the pre-cut fabric to ensure that the fabric was fully covered with blood. The fabric samples were then left for 72 hours at an average temperature of 26ºC to 29ºC for drying. These fabric samples were then attached to pieces of plywood and exposed to extreme sunlight and heavy rain in the three different locations for a period of 7 days. The sample preparation was based on a previous study [9] with minor modifications.

Sample Collection

Sample collection was performed daily for one week. Each day, 2cm x 2cm pieces were cut from the bloodstained fabrics and placed in separate paper bags. Temperature and humidity at the time of collection were recorded. The collected samples were preserved in the freezer at -20°C prior to the extraction process.

DNA Extraction

DNA extraction of the bloodstained samples was performed using a NEXprep™ NexK-3100 Genomic Mini Kit (Blood DNA Mini Kit, 100 prep). All the solution and extraction steps were performed according to the manufacturer's instructions.

DNA Quantification, µ

The concentration of DNA in each of the samples was measured by BioPhotometer Plus by Eppendorf. The wavelengths used for this measurement were 230, 260 and 280 nm. About 5 μL final extractant was pipetted into a 1.5 mL tube and diluted with 55 μL of distilled water, then placed in a Uvette cuvette. The Uvette was placed on the BioPhotometer Plus and the concentration of DNA for the sample was noted. The wavelength used for this measurement was 260 nm for nucleic acids detection, 280 nm for proteins and phenol and 230 for absorption of carbohydrates. The ratio of A_{260}/A_{280} represents the purity of the DNA contained in the fabric sample from a range of 1.8 to 2.0, and if the value is less than 1.8, the DNA in the fabric sample is not pure. The ratio of A_{260}/A_{230} represents the contamination of the DNA contained in the fabric sample and this value must be more than 2.0. A value of less than 2.0 indicates that the sample has been contaminated [10].

RESULTS AND DISCUSSION

DNA evidence found at a crime scene is often treated as crucial evidence to solve the crime. Biological fluids such as blood, semen or saliva on fabrics like clothing or bedding items are one of the most important evidentiary materials from a crime scene [11]. The survival of DNA evidence depends on several factors and environmental conditions as heat, humidity, chemicals, microorganisms and moisture can contaminate the DNA and make DNA analysis difficult [5]. Based on this study, when the fabric samples were exposed to different environmental conditions such as rain and sunlight, the colour of the blood on the fabric samples became a light brown. The colour of the blood and the structure of the fabric samples were observed day by day. The results showed that the fabric shrunk and hardened because of the drying blood and the amount of sunlight it was exposed to (Figure 1).

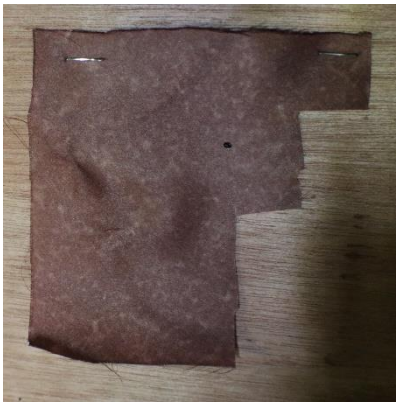

Figure 1. The condition of the fabric after 7 days of exposure in an abandoned building

The temperature in the abandoned building for this study from day 1 to day 7 was in the range of 26.0 to 33.0 ℃. As this was not at room temperature, the DNA did degrade, but not as fast as for the samples in the jungle and at the construction site. The humidity level was around 31 % to 49 %, indicating that the condition inside the building was dry. Temperature and humidity are known to play both physiological and evidential roles in DNA contamination. DNA is extremely sensitive to elevated temperatures over time. Low temperatures are well known for preserving DNA in samples [12]. Based on Table 1.1(a), the concentration of DNA retained by fabric in an abandoned building was much higher when compared to the concentration of DNA retained by fabric in the jungle and at the construction site. Table 1.1(b) shows that crepe was able to retain DNA for a long time when exposed to conditions in the abandoned building, as the difference between the initial and the final values of the DNA purity calculated was the smallest, at 0.28. The fabrics that had the poorest ability to retain DNA were valentro and georgette, as these two fabrics showed large differences in DNA purity values between the initial and the final days when placed in the abandoned building area. Overall, it can be seen from Table 1.1(c) that DNA contamination started to occur on valentro and georgette on day 4, when the contamination values went below 2.0. However, for crepe, the DNA was not contaminated until day 6, when the contamination value was 2.06.

Table 1.1(a). Concentration of DNA retained from fabrics exposed to an indoor abandoned building area for 7 days

Type of Fabric	Environmental conditions: Abandoned building							
	DNA Concentration (ng/μL)							
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Satin	86.8	84.6	73.2	65.7	63.1	51.2	41.1	29.4
Chiffon	88.1	72.5	68.2	63.7	54.6	49.1	42.1	29.7
Linen	100.8	95.6	83.2	72.7	66.1	57.2	46.1	35.4
Polyester	82.6	72.5	69.2	55.5	49.1	38.2	26.8	21.3
Cotton	98.1	82.3	76.2	70.7	61.1	52.2	42.1	31.7
Crepe	195.4	169.8	132.8	106.8	84.7	65.4	40.1	25.9
Argenti	164.9	135.5	97.3	84.5	61.6	49.7	25.1	17.4
Valentro	98.7	74.5	58.3	42.7	21.8	16.8	12.6	9.8
Lycra	123.2	106.5	74.3	59.1	44.8	28.7	16.7	10.9
Georgette	90.5	77.3	42.7	38.8	23.8	17.9	13.8	9.1

Table 1.2(b). Purity of DNA retained from fabrics exposed to an indoor abandoned building area for 7 days

Type of Fabric	Environmental conditions: Abandoned building							
	DNA Purity ($A_{260/280}$)							
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Satin	1.93	1.91	1.89	1.86	1.84	1.80	**1.76**	**1.62**
Chiffon	1.95	1.93	1.91	1.89	1.87	1.87	**1.78**	**1.66**
Linen	1.95	1.93	1.91	1.89	1.87	1.87	**1.78**	**1.66**
Polyester	1.93	1.91	1.89	1.86	1.84	1.80	**1.76**	**1.62**
Cotton	1.95	1.93	1.91	1.89	1.87	1.87	**1.78**	**1.66**
Crepe	2.04	2.00	1.97	1.93	1.88	1.85	1.80	**1.76**
Argenti	1.97	1.95	1.90	1.86	1.84	1.81	**1.71**	**1.35**
Valentro	1.98	1.89	1.83	1.80	**1.72**	**1.54**	**1.15**	**0.76**
Lycra	1.93	1.87	1.85	1.83	1.80	**1.76**	**1.69**	**1.37**
Georgette	1.91	1.85	1.83	1.81	**1.71**	**1.48**	**1.25**	**0.96**

* If the value is less than 1.8, the extracted DNA from the fabric samples is not pure

Table 1.3(c). Contamination of DNA extracted from fabrics exposed to an indoor abandoned building area for 7 days

Type of Fabric	Environmental conditions: Abandoned building							
	DNA Contamination ($A_{260/230}$)							
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Satin	2.30	2.20	2.18	2.16	2.12	2.00	**1.97**	**1.73**
Chiffon	2.40	2.20	2.19	2.17	2.12	2.12	**1.97**	**1.73**
Linen	2.20	2.20	2.19	2.17	2.12	2.12	**1.97**	**1.73**
Polyester	2.20	2.20	2.18	2.16	2.12	2.00	**1.97**	**1.73**
Cotton	2.20	2.20	2.19	2.17	2.12	2.12	**1.97**	**1.73**
Crepe	2.43	2.40	2.35	2.29	2.20	2.14	2.06	**1.92**
Argenti	2.32	2.28	2.24	2.21	2.18	2.02	**1.96**	**1.67**
Valentro	2.35	2.29	2.18	2.04	**1.94**	**1.72**	**1.37**	**0.93**
Lycra	2.35	2.22	2.17	2.11	2.03	**1.94**	**1.73**	**1.58**
Georgette	2.27	2.19	2.10	2.09	**1.96**	**1.60**	**1.47**	**1.15**

* If the value is less than 2.0 the sample is considered to have been contaminated

Table 1.2(a) shows that the DNA concentrations in all 10 types of fabrics placed in the jungle area were the lowest compared to the other two locations. According to Table 1.2(b), from day 4 of jungle exposure, the ratio for DNA purity was below 1.8 for most of the fabrics, indicating that the DNA was not pure. Table 1.2(c) also reveals that most of the DNA on the fabrics were already contaminated starting from day 4 as there was fungal growth on the fabrics (Figure 2). The results also indicate that the DNA contamination occurred faster when the fabrics were exposed to jungle conditions. The fabric that was able to retain DNA the longest when placed in the jungle was crepe, as shown by the value of DNA purity until day 5. The fabric that had the least ability to retain DNA was georgette. This was proven by the ratio of DNA purity obtained on day 3 which was 1.79, indicating that the DNA was already contaminated. Apart from this, the bloodstained fabrics exposed to the jungle area and construction site had higher humidity levels and were exposed to direct sunlight compared to the abandoned building. The temperatures recorded in the jungle area were between 23.0 ºC to 35.0 ºC and the humidity range was around 52 % to 96 %. The higher percentage of humidity shows that the area experienced high rainfall and a large amount of sunlight. Rainfall was recorded on day 1, day 2, day 4 and day 6.

Figure 2. Condition of the fabric after 7 days of exposure to the outdoor jungle area

Table 1.2(a). Concentration of DNA retained from fabrics exposed to the outdoor jungle area for 7 days

Type of Fabric	Environmental conditions: Jungle							
	DNA Concentration (ng/μL)							
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Satin	86.4	69.6	63.2	52.7	44.1	36.2	21.1	14.2
Chiffon	88.1	71.6	64.0	53.7	44.5	36.9	23.1	17.2
Linen	100.7	87.6	79.2	69.7	56.1	49.2	39.1	28.3
Polyester	82.6	63.5	52.2	42.7	39.1	29.2	19.1	11.3
Cotton	98.1	77.6	69.2	59.7	46.1	39.2	28.1	22.3
Crepe	195.4	154.8	117.8	82.8	63.7	35.4	20.1	13.2
Argenti	164.9	113.5	86.3	61.6	49.7	25.9	16.8	7.4
Valentro	98.7	74.5	58.3	42.7	21.8	16.8	9.6	5.8
Lycra	123.2	96.5	74.3	55.1	24.8	18.7	12.7	8.9
Georgette	90.5	67.9	32.7	28.8	13.8	7.90	3.80	1.10

Table 1.2(b). Purity of DNA retained from fabrics exposed to the outdoor jungle area for 7 days

Type of Fabric	Environmental conditions: Jungle							
	DNA Purity ($A_{260/280}$)							
	Day 0	Day1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Satin	1.95	1.88	1.80	1.80	**1.68**	**1.50**	**1.48**	**1.42**
Chiffon	1.92	1.88	1.80	1.80	**1.62**	**1.58**	**1.52**	**1.35**
Linen	1.95	1.88	1.80	1.80	**1.65**	**1.58**	**1.52**	**1.46**
Polyester	1.95	1.88	1.80	1.80	**1.65**	**1.50**	**1.48**	**1.42**
Cotton	1.92	1.88	1.80	1.80	**1.62**	**1.58**	**1.52**	**1.35**
Crepe	2.04	1.97	1.93	1.88	1.86	1.80	**1.65**	**1.25**
Argenti	1.97	1.93	1.86	1.84	1.81	**1.77**	**1.45**	**1.03**
Valentro	1.98	1.89	1.84	1.80	**1.72**	**1.54**	**1.15**	**0.76**
Lycra	1.93	1.89	1.86	1.80	**1.73**	**1.56**	**1.35**	**1.17**
Georgette	1.91	1.87	1.83	**1.79**	**1.26**	**0.73**	**0.45**	**0.06**

* If the value is less than 1.8, the extracted DNA from the fabric samples is not pure

Table 1.2(c). Contamination of DNA extracted from fabrics exposed to the outdoor jungle area for 7 days

Type of Fabric	Environmental conditions: Jungle							
	DNA Contamination ($A_{260/230}$)							
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Satin								
Chiffon	2.20	2.20	2.20	2.09	**1.96**	**1.84**	**1.83**	**1.60**
Linen	2.20	2.20	2.20	2.13	**1.96**	**1.84**	**1.83**	**1.79**
Polyester	2.20	2.20	2.20	2.13	**1.96**	**1.76**	**1.75**	**1.73**
Cotton	2.20	2.20	2.20	2.09	**1.96**	**1.84**	**1.83**	**1.60**
Crepe	2.43	2.32	2.27	2.21	2.15	2.03	**1.83**	**1.56**
Argenti	2.32	2.27	2.21	2.18	2.02	**1.91**	**1.72**	**1.24**
Valentro	2.35	2.29	2.21	2.04	**1.94**	**1.72**	**1.37**	**0.97**
Lycra	2.35	2.28	2.17	2.08	**1.89**	**1.78**	**1.63**	**1.38**
Georgette	2.27	2.23	2.07	**1.99**	**1.57**	**0.91**	**0.64**	**0.15**

* If the value is less than 2.0 the sample is considered to have been contaminated

The DNA purity values for all fabrics exposed to the construction site were lower compared to the fabrics exposed to the abandoned building. Based on Table 1.3(a), linen had the highest DNA concentration among the fabrics tested at the construction site. Also, the data in Table 1.3(b) for DNA purity shows that crepe still gave pure DNA on day 4, followed by satin, chiffon, linen, polyester and cotton. The fabrics that had the least ability to retain DNA were valentro, georgette and lycra as shown in Table 1.3(c). The temperature at the construction site from day 1 to 7 was in the range of 24ºC to 34.0 ºC, while the humidity was between 51 to 94%. Contamination and degradation can destroy biological evidence. Contamination and degradation of DNA can be caused by microorganisms, temperature, heat, moisture, and other living and non-living factors [13].

Table 1.3(a). Concentration of DNA retained from fabrics exposed to the outdoor construction site for 7 days

Type of Fabric	Concentration Environmental conditions: Construction Site							
	DNA (ng/µL)							
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Satin	86.7	64.0	60.2	49.1	43.1	30.2	18.6	10.4
Chiffon	88.1	68.6	60.3	49.7	42.5	31.2	25.7	13.2
Linen	100.7	82.5	73.5	58.6	49.7	42.1	33.5	23.5
Polyester	82.6	59.2	48.2	32.1	28.3	21.6	15.7	10.3
Cotton	98.1	72.8	61.7	53.2	40.2	34.3	22.5	19.2
Crepe	195.4	136.7	104.8	72.8	53.1	25.7	17.9	7.5
Argenti	164.9	97.5	68.3	49.6	25.7	16.9	6.8	2.4
Valentro	98.7	64.5	48.9	27.7	16.8	9.3	5.6	1.7
Lycra	123.2	76.5	54.4	25.1	18.1	15.7	12.7	8.9
Georgette	90.5	57.9	22.7	18.8	7.8	4.9	1.8	0.2

Table 1.3(b). Purity of DNA retained from fabrics exposed to the outdoor construction site for 7 days

Type of Fabric	Environmental conditions: Construction Site							
	DNA Purity ($A_{260/280}$)							
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Satin	1.92	1.88	1.86	1.82	1.80	**1.63**	**1.52**	**1.46**
Chiffon	1.92	1.88	1.86	1.82	1.80	**1.63**	**1.52**	**1.46**
Linen	1.92	1.88	1.86	1.82	1.80	**1.63**	**1.52**	**1.46**
Polyester	1.92	1.88	1.86	1.82	1.80	**1.63**	**1.52**	**1.46**
Cotton	1.92	1.88	1.86	1.82	1.80	**1.63**	**1.52**	**1.46**
Crepe	2.04	1.93	1.89	1.83	1.81	**1.72**	**1.25**	**0.94**
Argenti	1.97	1.86	1.84	1.80	**1.77**	**1.45**	**1.03**	**0.52**
Valentro	1.98	1.86	1.83	**1.79**	**1.52**	**1.17**	**0.76**	**0.21**
Lycra	1.93	1.86	1.80	**1.73**	**1.61**	**1.43**	**1.20**	**0.98**
Georgette	1.91	1.86	1.82	**1.76**	**1.22**	**0.79**	**0.47**	**0.03**

* If the value is less than 1.8, the extracted DNA from the fabric samples is not pure

Table 1.3(c). Contamination of DNA extracted from fabrics exposed to the outdoor construction site for 7 days

Type of Fabric	Environmental conditions: Construction Site							
	DNA Contamination ($A_{260/230}$)							
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Satin	2.20	2.15	2.20	2.16	2.00	**1.89**	**1.76**	**1.66**
Chiffon	2.20	2.15	2.20	2.16	2.00	**1.89**	**1.76**	**1.66**
Linen	2.20	2.15	2.20	2.16	2.00	**1.89**	**1.76**	**1.66**
Polyester	2.20	2.15	2.20	2.16	2.00	**1.89**	**1.76**	**1.66**
Cotton	2.20	2.15	2.20	2.16	2.00	**1.89**	**1.76**	**1.66**
Crepe	2.43	2.26	2.20	2.15	2.09	**1.96**	**1.67**	**1.15**
Argenti	2.32	2.21	2.12	2.18	**1.91**	**1.72**	**1.24**	**0.69**
Valentro	2.35	2.21	2.14	**1.98**	**1.76**	**1.34**	**0.97**	**0.54**
Lycra	2.35	2.18	2.08	**1.91**	**1.78**	**1.59**	**1.42**	**1.07**
Georgette	2.27	2.20	2.08	**1.93**	**1.50**	**0.96**	**0.63**	**0.08**

* If the value is less than 2.0 the sample is considered to have been contaminated

The type of fabric also affects the penetration of bloodstains into the fabric. The degree of distortion of bloodstains observed on the fabric and the ability of the fabric to absorb the blood depends on its texture [14]. The presence of functional groups such as the O–H groups of cotton and rayon and the N–H groups of nylons and wool allow the formation of hydrogen bonds with nucleic acid chains, that result in strong intermolecular attractions. Polyesters and acrylics contain polar carbonyl and cyano groups, which permit relatively weaker dipole-dipole attractions with nucleic acid chains [11,15,16].

CONCLUSION

The interaction between blood and fabric is a controversial subject as the field of bloodstain analysis is still new. Much research has been done to evaluate the effects of cleaning products on the quantity and quality of extracted DNA from bloodstained cloth and interpret bloodstain patterns deposited on fabrics, compared to the work done to study the effects of different environmental conditions towards blood-stained fabrics. The findings of this research showed that the type of fabric with more persistent DNA when exposed to three different environmental conditions was linen for natural fabrics, and crepe for synthetic fabrics. The concentration of DNA retained by the fabric samples in an indoor abandoned building was higher compared to the concentration of DNA retained by the fabric samples at the construction site and in the jungle. The results of this study also showed that if the bloodstained fabrics were collected within 48 hours of the crime, there was a very high possibility of extracting pure DNA from all types of fabrics exposed to different environmental conditions. Forensic scientists should have a better understanding of biological materials and substrates, as well as the effect of environmental exposure on the DNA extraction analysis, in order to interpret test results more accurately.

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