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A preliminary study of STR profile assessment from bloodstains on clothes imitating real case conditions

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Abstract

The objective of this study was to assess the short tandem repeat (STR) profile of bloodstains on clothes subjected to real environmental conditions. Bloodstains of five individuals were prepared on three different types of clothes (jeans, spandex and cotton) and left outdoor for up to nine weeks. Samples were collected every week for Deoxyribonucleic acid (DNA) extraction and (STR) profile analysis. The quality of DNA profiles was evaluated from the number of detected allelic peaks. The DNA concentrations of bloodstains were highly variable; spandex (4.78-12.08 ng/µL), jeans (3.78-7.29 ng/µL) and cotton (4.38-14.18 ng/µL). The bloodstains on cotton which had been left for more than 5 weeks tended to give incomplete STR profiles. It is clear that outdoor exposure time had an effect on the loss of detected alleles. Interestingly, bloodstains on cotton tended to lose alleles more than those on jeans and spandex. These results supported that type of clothes also had an effect on STR analysis from bloodstains. From nine weeks of outdoor exposure, bloodstain samples on the three types of clothes had eventually shown less than 50% of the detected alleles.

Keywords: Bloodstains, DNA, Outdoor exposure, STR profile

1. Introduction

When a crime has occurred, bloodstains are a target of interest that can be used for Deoxyribonucleic acid (DNA) analysis. In forensic science, the analysis of human DNA is focused on polymorphic DNA sequence called microsatellite or short tandem repeat (STR). The more the STR loci are analyzed, the higher the power of discrimination between individuals will be. Therefore, commercial kits for 15 loci STR analysis plus sex determination, for example AmpF*l*STR® Identifiler® plus, PowerPlex® 16 System, are widely used in forensic DNA laboratory. The quality of the STR profile is considered from the appearance of allelic peaks, e.g. peak balance/imbalance, peak heights and areas and the presence of allelic dropout [1]. The STR profile with a bad quality cannot be used for comparison with those of the referenced DNA. Hence, the identification of the perpetrator by STR analysis cannot be achieved.

As the quality of the STR profile has an impact on the success of forensic DNA investigation, many studies have reported factors affecting the quality of the STR profile from blood. These include the methods used for DNA extraction [2], cleaning agents [3, 4], deposited substrates [5], presumptive test agents [6-8], storage temperature [9, 10] and UV exposure [11]. They found that the evidence's conditions either being contaminated by PCR inhibitors or being highly degraded by the environments can cause the failure of STR analysis. However, in comparison to the failure caused by PCR inhibitors, factors that can cause the degradation of evidence's DNA are of greater concern. This phenomenon is supported by the evidence that shows the relations between the numbers of STR allelic peaks and the degradation rate of evidence's DNA [12].

Although many studies on factors affecting the STR profile analysis from bloodstains have been reported, none was carried out under the real condition. In the real circumstance, evidences are exposed to uncontrolled conditions e.g. temperature, humidity and bacterial contamination. Therefore, this study was performed in order to examine the effects of real conditions on STR analysis from bloodstains on various types of clothes including cotton, spandex and jeans that had been left outdoor for a number of weeks. The DNA amounts and STR profile analysis of these samples were compared. The results of this study will be a guidance for forensic DNA analysis of bloodstains in the real condition.

2. Materials and methods

2.1 Bloodstains samples

This study was carried out under the permission from the Khon Kaen University ethics committee in human research (HE591369). Blood samples were withdrawn from five volunteers with no anti-coagulant added. Clothes including jeans, spandex and cotton were purchased from a department store. Clothes were washed, dried and then cut into A4 size. Bloods were dropped onto 20 spots (50 µL/spot) on each piece of cloth and left to dry before being placed outdoor for nine weeks. The blood spots (1 spot/person/cloth) were cut off every week using sterile scissors for further DNA analysis. Therefore, five replicates were examined at a time. Dried blood spots which had been cut off at the first date of experiment were used as controls. The experiment was performed during February-April 2017. The temperature and humidity were recorded every day.

2.2 DNA extraction and quantification

The DNA extraction was performed by using QIAamp DNA Micro Kit in accordance with the manufacturer's instruction with slight modification. The spots of bloodstains were cut into small pieces and then placed in the micro centrifuge tube. An aliquot (250 µL) of buffer ATL and 20 µL Proteinase K (20 mg/ml) were subsequently added. The tubes were then vortexed for 30 sec and then placed at 56°C for an hour. An aliquot (250 µL) of buffer AL was added and thoroughly mixed before being incubated at 70°C for 10 min. During incubation, the tubes were repeatedly inverted every 2 min. The tubes were spun at 13,000 rpm for 3 min. The supernatant was transferred to a column provided by the kit. The column was spun at 8,000 rpm for 1 min. The flow through was discarded and 500 µL of buffer AW1 was added into the column for washing. The column was spun down. This washing step was repeated twice. The DNA was then eluted from the column. The DNA amount was quantitated at 260 and 280 nm using Nanodrop spectrophotometer.

2.3 PCR amplification

The PCR amplification was performed by using AmpFlSTR® Identifiler® Plus. An amplification reaction comprising 2.5 µL Master mix, 1.25 µL primers and 1.25 µL diluted DNA sample (1 ng) was prepared. The reaction was thoroughly mixed and subsequently placed in the Thermocycler (Gene Amp® PCR 9700). The PCR conditions including an initial hold at 95°C for 11 min, followed by 28 cycles of denaturation step at 94°C for 1 min, an annealing step at 55°C for 1 min, an extension step at 72°C for 1 min, then a final elongation step at 60°C for 60 min followed by a final hold at 4°C were set up.

2.4 STR typing

A reaction mixture composed of 1 µL PCR product (or allelic ladder) and 9 µL of form amide: size standard mixture was prepared, heated at 95°C for 5 min and then placed on ice for 2 min. The samples were subsequently subjected to capillary using electro kinetic injection and the electrophoresis performed by ABI 3130 Genetic Analyzer was carried out. The DNA profiles were analyzed using GeneMapper® ID v3.2.1. The number of detected alleles from each of those samples were compared.

3. Results

3.1 Temperature and humidity

The temperature and humidity were measured during the experiment. Results are shown in Table 1. It can be seen that temperature and humidity fluctuated. The highest temperature (39.6 °C) was observed on week 6. On week 4, 6, 7 and 8 there was rain. However, the highest humidity was observed on week 7.

Table 1 The ambient temperature (minimum, maximum) and humidity during the experiment.

Week	Temperature (°C)		Humidity (%)	Rain
	Min	Max		
1	17.2	35.4	57.5	-
2	14.9	35	54.4	-
3	16.3	35.8	51.4	-
4	18.1	38.5	60	light rain on day 3 and 4
5	18.1	37.2	52.1	-
6	22	39.6	57.7	light rain on day 1
7	22.8	37.6	71.7	light rain on day 7
8	20.2	36.2	70.4	moderate rain on day 5
9	20.4	36.1	65.2	-

3.2 DNA concentration from bloodstains

The DNA amounts from bloodstains on spandex, jeans and cottons that had been left outdoor for nine weeks are shown in Table 2 and Figure 1. It is clear that outdoor exposure time had no effect on the amounts of DNA as the amounts of DNA extracted from bloodstains being left for three or four weeks were lower than those of the controls. On the contrary, the amounts of DNA from bloodstains exposed outdoor for five weeks were higher than those of bloodstains being left for nine weeks (spandex and jeans) and seven weeks (cottons). Interestingly, at the same period of exposure time (controls, 1w, 2w, 3w, 7w, 8w and 9w) the DNA amounts from bloodstains on cotton were higher than other clothes. In this case, it seems that types of clothes may have an effect on the amount of DNA extracted from bloodstains.

3.3 STR typing

The success of STR typing is considered from the full number of 32 detected alleles from 15 STR loci plus 1 amelogenin (Figure 2). These results are shown in Table 3 and Figure 3. It can be seen that one-week outdoor-exposed bloodstains still had all detected STR alleles (32 alleles). However, as the exposure time increased, the numbers of detected STR alleles tended to decrease falling to less than 50% (15/32) in the 9-week outdoor-exposed bloodstains. Interestingly, the reduction of the detected alleles presumably depended on the types of clothes. In comparison with spandex and jeans at the same period of exposure time, cotton was not a good substrate for STR profile examination from bloodstains because the numbers of alleles from bloodstains on cotton were less than those of spandex and jeans.

4. Discussion

DNA analysis of bloodstains mimicking real conditions has been examined in this study. Three different types of clothes including spandex, jeans and cottons were used as substrate for bloodstains. The DNA amounts and STR profiles considered from the number of detected alleles were examined from the bloodstain samples. For DNA extraction, we used silica membrane column-based method because it contains a rapid DNA purification step. Moreover, it has been reported that the STR profiles of samples extracted by silica membrane-based methods produce full profile with balanced peak, low noise and high reproducibility compared to other DNA extraction methods e.g. paramagnetic resin, Chelex resin and desalting [2].

The DNA concentrations from the bloodstain samples exposed outdoor at different periods of time varied indicating no correlations between outdoor exposure time and the amounts of DNA. This variation was probably caused by the contamination of bacteria on bloodstains as this study was conducted in the real environment. Hence, bacterial DNA was carried over during DNA extraction process resulting in the fluctuation of the DNA concentrations from bloodstains. To compare only the amounts of human DNA from bloodstains, the Quantifiler™ Human DNA Quantification kit could be an alternative method for the DNA quantification. This kit quantifies human DNA based on real-time PCR assay. Therefore, many studies have used this kit to quantify human DNA from forensic evidences [13]. Considering the clothe types, bloodstains on cotton mostly gave higher DNA concentration than others. It may possibly be explained by the fact that cotton is a natural fiber. It absorbs moisture rather than repels it. This promotes the growth of bacteria resulting in higher amounts of the extracted DNA.

The assessment of STR profiles from bloodstains on clothes were considered based on the numbers of the detected alleles. It can be seen that the numbers of the detected alleles from bloodstains on all clothes were less than 50% after 9 weeks of outdoor exposure. The reduction of the detected alleles may be caused by the degradation of DNA [12] or the decline of human DNA recovery [14] as shown by previous studies. In addition, studies on the effects of various chemicals to the quality of STR profiles had revealed that chemicals causing DNA degradation are the major cause of quality reduction [3,4,7]. The degradation of DNA in outdoor-exposed bloodstains may result from UV radiation. It has been reported that UV radiation can cause DNA damage in different ways e.g. base modification, strand breaks and photoproducts [15]. However, UV radiation is not only a major cause of DNA damage resulting in allelic drop-outs on the STR profile. Other factors including heat, light, humidity and the growth of microorganisms can also damage DNA. Microorganisms secrete DNA digesting enzymes. Humidity increases rehydration rate of DNA facilitating the diffusion of damaging agents [11]. These factors should especially be taken into consideration when outdoor-exposed bloodstains evidences were analyzed.

From this study, it can be seen that the DNA amount from bloodstains is not a key to the success of STR analysis since the DNA concentration could be constituted of not just human but also bacterial DNA. Previous study on DNA analysis from cigarette butts exposed outdoor had shown that the purity of DNA could be the potential parameter for STR analysis [16]. For the future work, it will be useful to examine this parameter in order to evaluate the success of STR analysis from bloodstains. In addition, the effect of variables temperature, humidity and bacterial activity needs to be dealt separately to be of use in practical situation.

Table 2 DNA concentration from bloodstains (n=5) on three different types of clothes exposed outdoor for 9 weeks.

Types of clothes	DNA concentration (ng/µL)									
	Control	1w	2w	3w	4w	5w	6w	7w	8w	9w
Spandex	5.65± 0.21	6.84± 0.28	4.78± 0.03	5.42± 0.06	5.60± 0.11	12.08± 0.08	11.68± 0.06	5.00± 0.17	5.48± 0.06	6.04± 0.08
Jeans	5.80± 0.28	7.29± 0.03	5.08± 0.20	5.10± 0.23	3.78± 0.08	6.62± 0.14	5.70± 0.14	7.74± 0.03	6.72± 0.08	6.02± 0.11
Cotton	7.70± 0.14	8.61± 0.01	14.18 ±0.17	5.94± 0.11	4.38± 0.23	9.80± 0.03	6.90± 0.31	8.62± 0.06	10.28± 0.23	11.12± 0.08

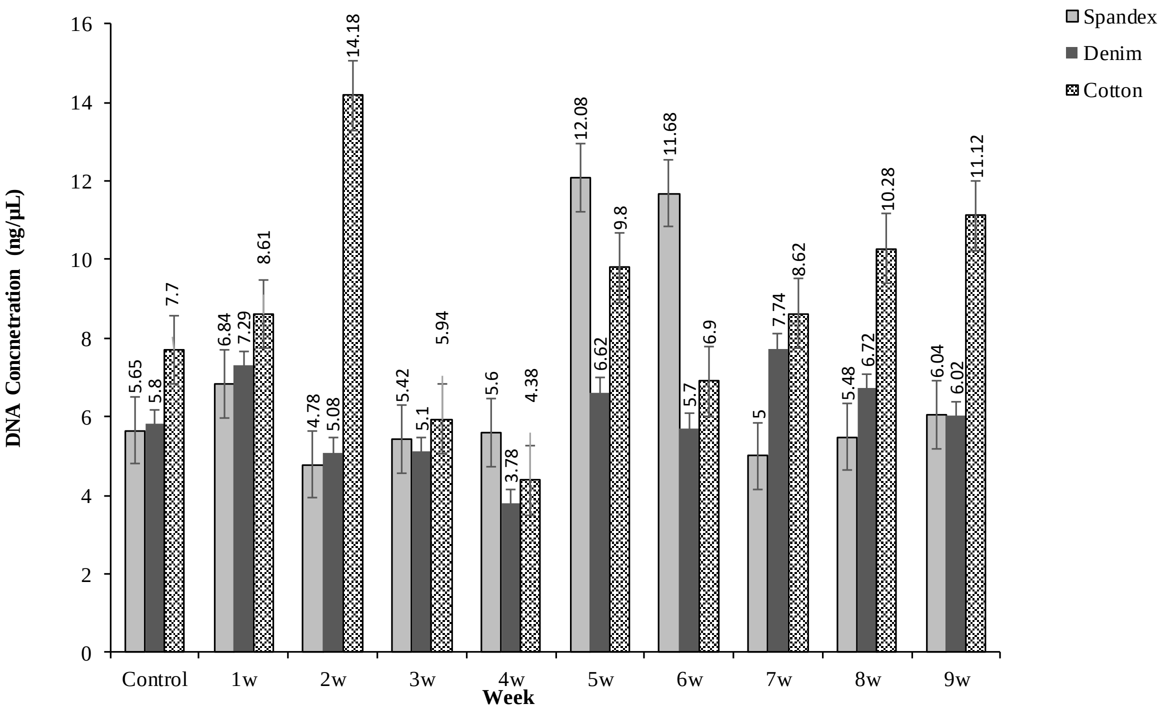

Figure 1 DNA concentrations from bloodstains on three different types of clothes exposed outdoor for 9 week.

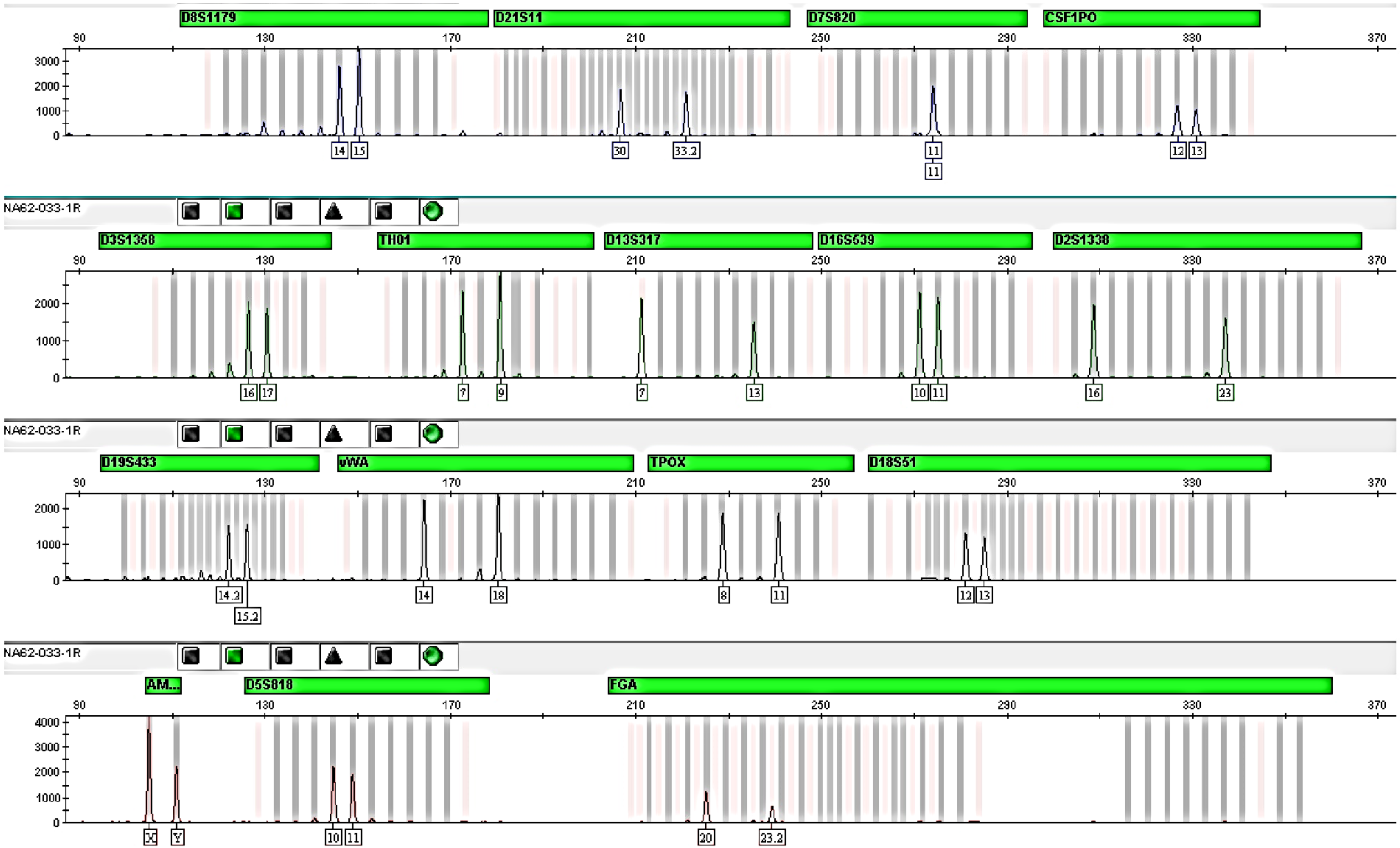

Figure 2 The success of STR analysis representing 32 alleles from 15 STR loci plus 1 amelogenin gene.

Table 3 The number of STR detected alleles amplified by AmpFlSTR® Identifiler® Plus from bloodstains (n=5) on clothes exposed outdoor.

Types of clothes	Numbers of detected alleles Control	1w	2w	3w	4w	5w	6w	7w	8w	9w
Spandex	32.00± 0.00	32.00± 0.00	31.60± 0.84	31.20± 1.78	32.00± 0.00	30.80± 2.68	31.60± 0.84	32.00± 0.00	20.40± 7.40	13.60± 2.19
Jeans	32.00± 0.00	32.00± 0.00	32.00± 0.00	32.00± 0.00	32.00± 0.00	32.00± 0.00	32.00± 0.00	32.00± 0.00	18.00± 1.41	14.40± 3.84
Cotton	32.00± 0.00	32.00± 0.00	29.60± 2.45	30.40± 2.60	31.20± 1.78	28.40± 4.09	23.20± 1.78	23.20± 3.34	12.80± 2.68	14.80± 4.14

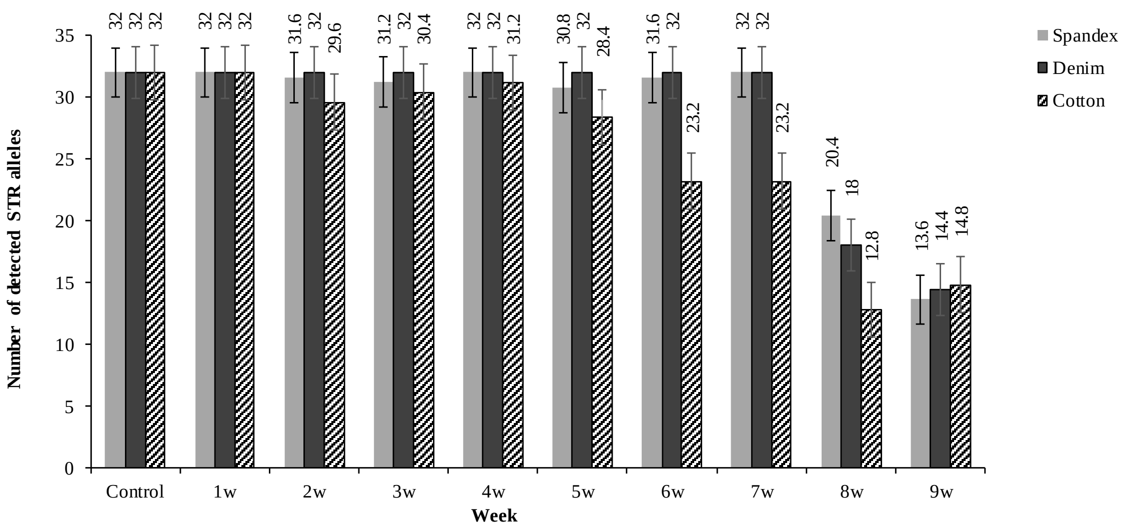

Figure 3 The numbers of detected STR loci amplified from bloodstains samples (n=5) on clothes exposed outdoor for up to 9 weeks.

5. Conclusions

This study has shown that the outdoor exposure time and types of clothes had no effect on the DNA amounts from bloodstains. On the other hand, these factors had an effect on the quality of STR profiles in which the number of detected alleles dropped to less than 50% in 9-week outdoor-exposed bloodstains. In addition, STR profiles from bloodstains deposited on cottons tended to lose some alleles more than those of bloodstains deposited on spandex or jeans.

6. Acknowledgement

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7. References

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