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The efficacy of luminol in detecting bloodstains that have been washed with sodium percarbonate and exposed to environmental conditions

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ABSTRACT

Blood evidence has a highly valuable role to play in crime investigation and crime reconstruction both in terms of DNA evidence and bloodstain pattern analysis (BPA). This paper presents the results of experiments that were designed to investigate the persistence and detectability of blood on clothing when exposed to different conditions and when washed in conjunction with sodium percarbonate (also known as active oxygen). Previous studies have demonstrated that the removal of traces of blood from denim and carpet is improved by the addition of sodium percarbonate, when compared with washing with detergent alone. In exploring this issue further, this study confirms that the efficacy of sodium percarbonate in removing bloodstains depends on the temperature of the wash cycle, the type of detergent used, drying time of blood and exposure of bloodstains to environmental conditions. The implications for the interpretation or blood evidence are considered, while the importance of continuing to develop an evidence base for the interpretation of blood evidence is emphasized.

1. Introduction

Blood evidence is frequently encountered in the forensic investigation of violent crime. It is a valuable form of evidence for forensic investigation purposes[1] owing to its capacity to provide DNA evidence[2], as well as to provide valuable intelligence and evidence from the interpretation of stain patterns (Bloodstain Pattern Analysis)[3–6]. Although DNA has limited chemical stability, research suggests that usable DNA for the purposes of analysis may be retained in dried bloodstains after several months, if not years, under adverse conditions[7] such as prolonged exposure to extreme temperature[2] and in substrates such as soil and asphalt[8–10].

The recovery of blood evidence can assist in the process of crime reconstruction. Its recovery is dependent on the detection of the presence of biological substances at crime scenes and on pertinent exhibits. Presumptive tests are frequently employed during the examination of a crime scene to detect any latent traces of blood. One of the most widely used presumptive tests is luminol, which exhibits chemiluminescence (in the form of a blue glow) when in contact with haemoglobin found in blood. While luminol is highly sensitive, previous research acknowledges that there are a number of environmental domestic and industrial substances that can induce a false-positive chemiluminescent reaction in the pres- ence of luminol. Such substances include soils, bleaches and vegetable compounds[11–17]. Only a few studies have explored substances that can suppress the luminol reaction and generate a false-negative result. For instance, recent research has demonstrated that cleaning products that contain sodium percarbonate can remove bloodstains so that presumptive and confirmatory tests are unable to detect the presence of blood[18,19]. While the occurrence of these false-negatives might be rare, the potential exists for crucial DNA evidence to go undetected.

Previous research has indicated that washing at high temperatures can cause blood to ‘fixate’ on fabric, thus making it difficult to remove[20,21]. However, there is a need for a sys- tematic study that examines the effectiveness of sodium percarbonate at removing blood- stains in combination with different types of detergents at different washing temperatures. This is particularly important because biological detergents contain enzymes that are designed to hydrolyze protein molecules and, therefore, have the potential to further degrade bloodstains[20]. The age of bloodstains is also an important parameter to explore in connection with the effects of sodium percarbonate given that

dried blood consistently produces a more intense chemiluminescence that lasts for a longer period of time[22]. Furthermore, while the resilience of blood to environmental conditions has been explored in connection with DNA analysis[2,7–10,12], less attention has been afforded to studying the potential for presumptive tests to detect blood that has been exposed to the environment.

It is increasingly being recognized that for robust and meaningful interpretation of phys- ical evidence, an empirical understanding of the dynamics of that evidence is crucial[23]. There is therefore, a need for empirical experimental studies that can offer insights into the behav- iour of different forms of evidence under different conditions. This can then provide the beginnings of an evidence base for the effective collection and interpretation of evidence when it is encountered during the course of a forensic investigation[23,24].

This paper presents the findings from a set of experiments designed to offer an insight into the effects of sodium percarbonate on the capacity to detect bloodstains. This paper considers the implications of the findings and will help to provide useful and practical infor- mation to aid the collection, analysis, interpretation and presentation phases of criminal investigations where detergents and/or sodium percarbonate may have been used to remove blood from clothing. The experiments in this study test a variety of bloodstained substrates washed under different conditions and tested with luminol. Luminol was chosen as it is regarded as 20 times more sensitive than any other blood detection test and it is also highly effective at detecting old and degraded bloodstains[22].

2. Materials and methods

Four experiments were designed to assess the ability of luminol to presumptively identify bloodstains that have been exposed to various conditions in combination with sodium percarbonate. A total of 660 samples of denim were used across experiments 1 and 3 including 132 control samples. In addition, 400 carpet samples were used across experiments 2 and 4 including 80 control samples. Denim and carpet were selected as these materials may be routinely encountered at crime scenes. The breakdown of samples into conditions for each experiment is described below and summarized, along with the results of the lumi- nol tests in Tables 1–6.

2.1. Experiment 1

Experiment 1 was designed to test the influence of washing bloodstained

denim samples with biological and non-biological detergents at different wash temperatures (10 °C, 40 °C and 60 °C) with and without sodium percarbonate. In order to assess the variability of the results, 10 samples were tested per condition with the inclusion of two control samples. Overall, 120 samples and 24 control samples were used.

2.2. Experiment 2

Experiment 2 was designed to test the same processes as Experiment 1 but for carpet sam- ples. In order to imitate normal washing conditions, carpet samples were scrubbed by hand using 10 °C or 40 °C washing temperature with and without sodium percarbonate. Instead of using biological and non-biological detergent (which is predominately used to wash clothes), a neutral detergent was incorporated into this experiment. Research suggests that a neutral detergent (washing up liquid), is more proficient at removing bloodstains in com- parison with normal carpet detergents[25]. In addition, neutral detergents do not contain any corrosive chemicals (such as hydrogen peroxide or sodium percarbonate) which may affect the results[25]. Ten samples were tested per condition with the inclusion of two control sam- ples. In total, 40 samples and eight control samples were used.

2.3. Experiment 3

Experiment 3 was designed to test the efficacy of sodium percarbonate in removing aged bloodstains from denim. Bloodstain samples were divided into two sets – the first set of

Table 1. The effect of the presence of sodium percarbonate across different washing conditions.

Table 2. the effect of the presence of sodium percarbonate across different washing temperatures.

Table 3. the effect of sodium percarbonate on denim exposed to laboratory conditions.

Table 4. the effect of sodium percarbonate on denim exposed to environmental conditions.

Table 5. the effect of sodium percarbonate on carpet exposed to laboratory conditions.

Table 6. the effect of sodium percarbonate on carpet exposed to environmental conditions

bloodstains were allowed to dry in laboratory conditions for 0, 1, 4, 7 and 14 days and the second set were exposed to outdoor environmental conditions for 1, 4, 7 and 14 days. Once each bloodstain had been allowed to dry for the allocated time period, the denim samples were subsequently washed with or without sodium percarbonate at: 10 °C, 40 °C or 60 °C with biological or non-biological detergent. The samples were allowed to dry post-wash for a further day and subsequently tested with luminol. Five samples were tested per condition with the inclusion of one control sample per condition. In total, 540 denim samples and 108 control samples were used. With respect to the environmental conditions, the outside tem- perature between days 1 and 7 ranged between 15 °C and 22 °C with light to heavy rainfall.

Between 7 and 14 days, the temperature increased substantially, subjecting bloodstains to temperatures between 25 °C and 30 °C. The ultraviolet index, which measures the strength of the ultraviolet radiation from the sun, revealed a high UV exposure of >7.

2.4. Experiment 4

Experiment 4 was designed to test the efficacy of sodium percarbonate in removing aged bloodstains from carpet. Bloodstained samples were divided into two sets – the first set of bloodstains were allowed to dry in laboratory conditions for 0, 1, 4, 7 and 14 days and the second set were exposed to outdoor environmental conditions for 1, 4, 7 and 14 days. Once each bloodstain had been allowed to dry for its allocated period of time, the carpet samples were hand-washed with a neutral detergent at 10 °C or 40 °C washing temperature. The samples were allowed to dry (post-wash) for a further day and subsequently tested with luminol. A total of five samples were tested and one control sample per condition. In total, 360 carpet samples and 72 control samples were used.

2.5. Sample preparation

Each bloodstain was prepared using 0.3 ml of fresh porcine blood with dipotassium ethylenediaminetetraacetic acid (EDTA) added to prevent premature coagulation. This solution was immediately dispensed using a pipette onto 660 and 400 5 cm × 5 cm squares of white denim and white tufted carpet respectively. These were divided amongst the four experiments.

Control samples that did not contain traces of blood were used in order to test whether bloodstained fabrics would contaminate unstained fabrics; in total, two control samples per condition were used for Experiments 1 and 2 and one control sample for Experiments 3 and 4. A further five control samples were stained with 0.3 ml aliquots of blood without being subjected to washing procedures, in order to determine whether the luminol reagent was functioning correctly.

2.6. Reagent procedure

The luminol reagent was prepared using the Grodsky formula[26]: 25 g Na_2CO_3, 0.5 g 3-ami- nophthalhydrazide, 3.5 g sodium perborate and 500 ml of distilled water. The luminol reagent was sprayed onto post-wash fabric samples in a darkroom. The reagent test was repeated twice for each sample type. A positive result was recorded when the observers could see chemiluminescence which gradually dissipated after approximately 20 s. This method was used in order to distinguish between false-positive results, which are known to produce bright flashes of chemiluminescence when in contact with certain interfering substances. The result was considered negative when the luminol reagent failed to emit chemiluminescence on the fabric samples.

2.7. Camera procedure

Photos of the chemiluminescence were captured using a Fujifilm FinePix HS20EXR camera. The settings on the camera were adjusted in order to optimally capture images in low light conditions.

3. Results

The results are presented in Tables 1–6. The values represent the number of samples on which blood was successfully detected by the luminol test: 0 would indicate that no samples had detectable traces when exposed to the luminol reagent, whereas a score of 10 (for Experiments 1 and 2) or a score of 5 (for experiments 3 and 4) would indicate that the luminol test positively detected blood on all bloodstained samples within that particular condition. In all experiments, the control samples indicated that the luminol was functioning correctly. Furthermore, there was no evidence that the detection of blood could have resulted from contamination via the bloodstained fabrics.

3.1. Experiment 1

The data generated during Experiment 1 (Table 1) suggest that the presence of sodium percarbonate increased the effectiveness of the washing in removing detectable traces of blood from denim (34/60 samples reacting positively to the luminol test), when compared with washing solely with detergent (when all 60 samples tested positive after the experi- ment). With regard to the laundry detergents, the effects of biological and non-biological detergents with sodium percarbonate appeared to be very similar; washing the denim with biological detergent and sodium percarbonate removed detectable traces of blood in 14 of the 30 tests across temperature ranges, while non-biological detergent and sodium percar- bonate removed the detectable traces in 12 of the 30 tests.

The data in Table 1 also suggest that without the incorporation of sodium percarbonate, washing temperature had little effect on the removal of bloodstains; with all 60 denim sam- ples providing a positive reaction to the luminol test after washing. By contrast, no samples washed with non-biological detergent and sodium percarbonate at 60 °C provided a positive reaction to the luminol test, while only two samples washed with biological detergent at 60 °C reacted positively.

3.2. Experiment 2

The data generated during Experiment 2 (Table 2) indicate that the addition of sodium percarbonate to the wash with neutral detergent had no effect at removing detectable traces of blood from the carpet swatches when washed at 10 °C and 40 °C, with all samples regis- tering a positive result to the luminol test after washing.

3.3. Experiment 3

3.3.1. Aged versus fresh bloodstains and environmental versus laboratory bloodstains

Referring to the data presented in Tables 3 and 4, there did not appear to be discernible trends with respect to the length of the period over which stains were left to dry and the presence of detectable traces of blood. It does appear that, generally, drying (especially in environmental conditions) before washing encouraged the removal of detectable traces of blood. Further drying, however, had a limited effect.

When comparing the data for stains left in laboratory conditions (Table 3) and environ- mental conditions (Table 4) it is evident that, generally, the samples exposed to environ- mental conditions exhibited a greater reduction

in the presence of detectable blood under equivalent conditions.

3.4. Experiment 4

3.4.1. Aged versus fresh bloodstains and environmental versus laboratory bloodstains

The results presented in Table 5 indicate that there was some reduction in the presence of detectable levels of blood given drying times of more than one day under the laboratory conditions. All five samples were positively detected by the luminol test after 0 or 1 day drying times. However, when left for 4, 7 and 14 days, some samples did not register a positive test result.

Comparing Table 5 (laboratory conditions) with Table 6 (environmental conditions), it is evident that there were greater reductions in the presence of detectable blood on the carpet samples when exposed to the environmental conditions (given equivalent drying times and washing conditions).

4. Discussion

This series of experiments was designed in order to investigate the persistence and detect- ability of blood when exposed to different conditions and washed in conjunction with sodium percarbonate. As noted by Castello[18] and substantiated by the experiments con- ducted in this study, sodium percarbonate is more effective than a detergent alone in remov- ing traces of blood in denim and carpet. However, in contrast to the findings presented by Castello[19] this study has demonstrated that the removal of blood may also be contingent on a number of variables such as the washing temperature, the type of detergent used, the drying time of blood and exposure of bloodstains to environmental conditions.

4.1. Detergent

Across all the experiments explored in this paper, both biological detergent and non- biological detergent were not able to remove traces of blood without the additional use of sodium percarbonate. When sodium percarbonate was added, washes that were carried out with biological and non-biological detergent yielded very similar results in terms of the removal of detectable blood.

4.2. Washing temperature

Previous research by Fijan[27] has suggested that cold water is more proficient

at removing bloodstains than hot water. By contrast, the results of this experimental work suggest that a combination of sodium percarbonate and a washing temperature of 60 °C can be expected to remove detectable traces of blood on denim to a greater extent than cooler temperatures. The high number of bloodstained denim samples that provided positive luminol results when washed at 10 °C – 8/10 and 5/10 samples and 40 °C – 10/10 and 9/10 samples could be partly explained by the inefficiency of sodium percarbonate to dissolve easily in cold water and it is possible that it was not acting optimally in these cooler conditions. Experiment 2 also demonstrated similar findings with all bloodstained carpet samples providing positive luminol test results (40/40), this further strengthens the argument that sodium percarbonate only works effectively at high temperatures. Alternatively, previous research suggests that deeper porous surfaces (such as carpet) are able to retain more traces of blood, due to the extra surface layer acting as a protective barrier against washing and chemical agents such as sodium percarbonate[41].

4.3. Aged blood

It has been suggested that bloodstains left to dry for longer periods of time tend to bind the blood to the substrate, making them more difficult to remove[42]. Yet the findings from this study suggest that there may be some reduction in the detectability of blood if stains on denim and carpet are left to dry before washing, particularly under environmental con- ditions. These observations were surprising given that luminol has been shown to be more effective at detecting aged bloodstains as opposed to fresh[22].

4.4. Collection and interpretation

The findings of this study suggest sodium percarbonate is able to remove detectable traces of blood from denim and from carpet under certain conditions (high washing temperature and after exposure to environmental conditions). These findings may be of use to investi- gations where it is suspected that sodium percarbonate may have been used to wash or remove bloodstains and has subsequently resulted in a negative luminol test. As this paper has demonstrated, a negative presumptive test does not negate the presence of blood and it is potentially worthwhile to test suspected items and/or undertake additional analysis on such exhibits as it is possible that some traces of blood may still be recoverable. It is important to note that there are inevitable contextual issues that should be taken into account when assessing these results due to the nature of this form of experimental study.

An anticoagulant was used to preserve the blood utilized in this study as a necessary requirement of laboratory conditions. Blood encountered in forensic casework will not have been subject to such treat- ment and thus further studies are necessary to observe the behaviour of blood in the absence of an anticoagulant. In addition, it is recognized that in a laboratory setting it is possible to account and control for extraneous variables. In casework there will often be other biological substances such as saliva and semen present in addition to blood evidence and this could affect the sensitivity of the luminol reagent.

Porcine blood has been cited by some as a reasonable alternative to human blood[28]. However, the results in this study should be validated through replicate experiments using human blood. Future studies should also control the height at which blood is applied to substrates, as this may have affected the persistence and stain diameter of the blood used in this study. Furthermore, the fabrics used in this study had not been previously washed before staining. New fabrics are often subjected to finishing treatments, chemical treatments and heat treatments and the interaction of these with luminol is not well understood. As a consequence, future research should consider washing new fabrics at least once before applying blood. This would also increase the external validity of future experimental findings.

Notwithstanding these issues, the findings from this study present valuable empirical find- ings that develop the findings of previous work and highlight the importance of careful and thorough examination of forensic casework when using luminol as a presumptive testing method.

5. Conclusion

The preliminary experiments presented in this paper indicate that sodium percarbonate is more effective in removing significant traces of blood when compared with washing blood- stains solely with washing detergent, which proved ineffective at removing detectable blood. Most importantly, this paper demonstrates empirically for the first time in the published literature that the effectiveness of sodium percarbonate can be enhanced when other con- ditions (such as washing temperatures, drying time and environmental elements, etc.) are incorporated into the cleaning procedures utilized. These findings are of value for the col- lection and analysis of exhibits with suspected blood staining in forensic investigations. They also represent a contribution to an evidence base for the robust interpretation of blood evidence, thereby aiding the process of crime reconstruction.

Disclosure statement

No potential conflict of interest was reported by the authors.

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