تأثير بعض المنظمات الكيميائية وطرق غسيل بقع الدماء الجافة
على البصمة الوراثية الجسدية

مجدي حسن وآخرون

الدليل المادي هو وسيلة فعالة في ربط الجناة بمسرح الجريمة، والكشف عنه من الإجراءات اللازمة لاستكمال باقي التحقيقات. والدم هو أحد الأدلة المادية الأكثر شيوعاً في جرائم العنف ويعتبر مصدرًا مهمًا للمعلومات التي تكون حاسمة حين يعثر عليه في مسرح الجريمة وبعد تحليل الطب الشرعي.

وقد أجريت هذه الدراسة لتفعّل على تأثير بعض المنظمات الكيميائية على بقاء الحمض النووي الوراثي في بقع الدموية المغمولة بدوياً ومدى تأثير تلك المنظمات على ظهور السمات الوراثية الجسدية. وتضمنت الدراسة استخلاص الحمض النووي لعدد من العينات بعد غسلها بالماء فقط، والصابون (الأبيض)، وهيبوكسكلات الصوديوم (الكلوركس)، والصابون وهيبوكسكلات الصوديوم معاً وتم التحليل الكمي للعينات وعمل التكثير لمواقع السمات الوراثية، وبعد ذلك تم تحديد السمات الوراثية باستخدام جهاز التحليل الجيني.

وقد أظهرت الدراسة أن عينات البقع الدموية المغمولة بالماء العادي لم تتآثر في محتواها، وعينات البقع الدموية المغمولة بالصابون يدوياً تتأثرت من حيث محتوى السمات الوراثية لبعض العينات، أما العينات التي تم غسلها بالكلوركس نتج عنها فقد كامل لبعض السمات الوراثية لبعض العينات أما العينات التي تم غسلها بالصابون والكلوركس نتج عنها الاختفاء التام للسمات الوراثية لبعض العينات. وتعتبر الدراسة بعده إملاً لأثر دموى موجود على ملابس قد تعرضت للغسيل بقصد إخفاء معالم الجريمة.

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7- Creamer, JL.; Quickenden, TI.; Crichton, LB.; Robertson, P. and Ruhayell, RA.: Attempted Cleaning of Bloodstains and its Effect on the Forensic Luminol Test. Luminescence 2005; 20, pp. 411-3.


References


3- Passi, N.; Garg, RK.; Yadav, M.; Singh, RSS. And Kharoshah, MA.,: Effect of Luminol and Bleaching Agent on the Serological and DNA Analysis From Bloodstain. Egyptian Journal of Forensic Sciences - June 2012, 2, pp. 54–61


And see also:

5- Lee, HC.; Gaenslen, RE.; Pagliaro, EM.; Buman, MB.; Berka, KM. and Keith, TP.,: The Effect of Presumptive Test, Latent Fingerprint and Some Other Reagents and Materials on Subsequent Serological Identification, Genetic Marker and DNA Testing in Bloodstains. J. Forensic Ident, 1989, 39, pp. 331–50.


And see also:
- Fregeau, CJ.; Germain, O. and Fourney, RM. Fingerprint Enhancement Revisited and the Effects of Blood Enhancement Chemicals on Subsequent Profiler Plus_
Fig (7) STR genotyping of DNA isolated from bloodstain washed by Clorox showing drop alleles in (D7S820, and D18S51).

Fig (8) STR genotyping of DNA isolated from bloodstain washed by soap and Clorox together showing drop alleles in: (D21S11, D7S820, CSFIPO, D13S317, D16S539, D2S1338, D18S51 and
Fig (5) STR genotyping of DNA isolated from bloodstain washed by water.

Fig (6) STR genotyping of DNA isolated from blood stain washed by soap showing drop alleles in cells (D7S820, CSFIPO and D18S51).
Fig (3) Identifiler allelic ladder

Fig (4) STR genotyping of DNA isolated from control blood sample
Fig(1) Showing blood crystal after (A) washing by water (B) washing by soap (C) washing by clorox

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (ng/μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unw</td>
<td>57</td>
</tr>
<tr>
<td>Ww</td>
<td>88</td>
</tr>
<tr>
<td>Aw</td>
<td>110</td>
</tr>
<tr>
<td>Cw</td>
<td>126</td>
</tr>
<tr>
<td>C+Aw</td>
<td>203</td>
</tr>
</tbody>
</table>

Unw = unwashed sample  
Ww = water washed  
Aw = soap washed  
Cw = clorox washed
In majority of the cases complete autosomal STR profile was obtained from water or detergent washed blood stains, whereas bleach-washing of blood stains resulted in the loss of 2-3 autosomal STR loci.

Blood stain washed with soap and Clorox together, produced partial human DNA profile.

Autosomal STR loci D7S820, CSF1PO and D18S51 appeared to be the most affected by bleach and or detergent washing (Table 2). These results are in agreement with Passi, et al. (2012)\textsuperscript{(16)} who reported that bleaching agent, caused DNA degradation and it has the most adverse effect on the ability to obtain complete DNA profiles and also on the ABO blood grouping but it had very little effect on species determination and stated that significant degradation occurs. Harris, et al., 2006\textsuperscript{(17)} concluded that chlorine bleaches were the most effective in interfering with the DNA analysis. The bleaches contributed to DNA degradation and errors in peak shifting. However, they also relate that the general quality of the DNA was high. Coy, et al., 2005\textsuperscript{(18)} and Hochmeister, et al., 1999\textsuperscript{(19)} discussed that cleaning agents have an effect on DNA analysis of blood stain.

As a conclusion washed blood stain can be detected, by DNA extraction and genotyping using standard laboratory procedure. STR Amplification kits comprising low number of loci, such as Minifiler, may yield reproducible and conclusive profiles resulting from detergent treated and bleached blood stains.

### Table (1) concentration of DNA extracted from blood stains

<table>
<thead>
<tr>
<th>Sample</th>
<th>Unwashed</th>
<th>Water wash</th>
<th>Ariel wash</th>
<th>Clorox wash</th>
<th>Ariel + Clorox wash</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>204.0</td>
<td>168.0</td>
<td>160.0</td>
<td>85.0</td>
<td>72.0</td>
</tr>
<tr>
<td>2</td>
<td>174.0</td>
<td>116.0</td>
<td>104.0</td>
<td>86.0</td>
<td>76.0</td>
</tr>
<tr>
<td>3</td>
<td>166.0</td>
<td>120.0</td>
<td>94.0</td>
<td>84.0</td>
<td>32.0</td>
</tr>
<tr>
<td>4</td>
<td>316.0</td>
<td>90.0</td>
<td>82.0</td>
<td>70.0</td>
<td>10.0</td>
</tr>
<tr>
<td>5</td>
<td>158.0</td>
<td>136.0</td>
<td>110.0</td>
<td>118.0</td>
<td>96.0</td>
</tr>
<tr>
<td>mean±SD</td>
<td>203±58.31</td>
<td>126±25.67</td>
<td>110±26.74</td>
<td>88±15.18</td>
<td>57±31.43</td>
</tr>
</tbody>
</table>
separated on 3130 Genetic Analyzer\textsuperscript{(14)} and genotyped using Gene mapper ID-X software version 1.0/1.1. Duplicate extractions were carried out, PCR was performed using Saiki, \textit{et al.}, 1988\textsuperscript{(15)} method.

\textbf{Control samples}
Profiles were obtained from blood samples prior to application and from stains prior to cleaning. These controls were used to help determine which contributory factor played the major effect in compromising the quality of the resultant profile.

\textbf{Results and discussion}
The preliminary and confirmatory tests were performed to identify the stains. The results are shown in Fig. 1. blood stain samples washed by water only were not affected and the crystals were observed. Blood stain samples washed by soap detergent were slightly affected and the crystals were observed in small amount, blood stain samples washed by Clorox were strongly affected and the crystals were observed in rare amount, where blood stain samples washed by soap and Clorox were strongly affected and the crystals were not observed after addition of Takayama reagent.

Out of all the cleaning agents bleach had the most deleterious effect on the quality of the DNA (Table 1 and Fig 2).

blood stains that were washed by water only were not affected in their content of DNA profile or genetic feature (Fig 3, 4 and 5) when compared to the treated blood stain.

blood stains of some samples that were washed by soap had been affected DNA in their content, profile and genetic feature. This is due to the fact that the soap compound contain chemical substances which affect DNA profile or the genetic feature (fig 6).

blood stains that were washed by Clorox had shown complete loss in the genetic feature of some samples and had affect the DNA profile. These occur because of the presence of alkaloid chemicals in the clorox which effect or destroy autosomal DNA (Fig 7).

The complete disappearance of the genetic features of blood stain that washed by Clorox and soap together occurred as a result of multiple effects of Clorox and soap (Fig 8).
until the stain was invisible. Then they ran standard DNA PCR analysis on those washed stain samples. They used commercial forensic automated analyzers and compared results. Partial to full Profiler Plus™ DNA profiles of the victim’s blood (a non-visible quantity) were obtained from areas of the suspect’s vehicle and from swabbings of each of the suspect’s hands (after they had been washed with running tap water) in the mock “crime scenes”.

Since very little information is available on the investigations of the effect of soap and bleaching agent (sodium hypochlorite) on serological markers and on qualitative and quantitative analysis of DNA from bloodstain. This study aimed to investigate the potential effect that these factors may have on DNA analysis.

Materials and methods

Samples, substrates and cleaning agents

Blood samples were obtained from 5 unrelated donors 2.5 ml from each one and the substrates used in this study were 25 pieces of white Saudi uniforms cloth (30% cotton and 70% silk). All bloodstains were prepared on square pieces measuring about 25 cm². 0.5 ml of blood were used to each one square of these pieces and these pieces divided into 5 groups. One group leaves as control without washing, and the other four groups were exposed to different ways of hand washing (2-3 min) first group washed only by water (3 L), second group by soap compound (Arial, 42.5 g), third group by chlorinated bleach (Clorox, 60 ml), and fourth one soap and chlorinated bleach together.

All substrates were UV irradiated for 20 min prior to stain application. Standard volume of blood was applied to each substrate and left to dry at room temperature (20-22°C) for 48 hour. Then hand cleaning was done and then left to dry again.

DNA extraction and detection

DNA from hand-washed detergent and bleached blood stains was extracted and quantified using standard molecular biology techniques. Autosomal STR loci were amplified using AmpFISTR Identifier Amplification kit. Amplified STR loci were size
Introduction
Blood stain is the most common evidence at the crime scene in one form or another. It may be encountered in different types of crime cases like murder, rape and assaults. They are required to be properly analyzed properly for their nature, species origin and individual characteristics. In an attempt to hide evidence, perpetrators can wash and destroy blood stains, making them undetectable to the naked eye and investigators cannot predict the conditions blood stains were subjected to before analysis. A wide variety of chemicals are used to affect the nature of blood stains and poses lot of problems in their analysis of blood stains.

Potential evidential material is often adulterated and rarely uncontaminated or of high quality. Advances in techniques and increased levels of sensitivity have meant that this need not prohibit confirming or negating a relationship between evidential and suspect material.

It has been shown that complete DNA profiles can be obtained from non-visible quantities of blood and the sensitivity of the Kastle–Meyer (KM) presumptive blood test makes the sourcing of such trace evidence possible\(^{(1)}\). The nature of the support or substrate on which the blood is suspended can itself introduce contaminants to the evidential material\(^{(2)}\). Bloodstains have also been deliberately removed from the crime scene by using a variety of bleaching agents\(^{(3)}\), luminol had no destructive effect, on confirmatory tests, species test or elution method for the detection of blood group antigens\(^{(4)}\) but again noted that it could seriously affect the electrophoretic typing of enzymes\(^{(5)}\). It has also been shown that following luminol treatment, DNA can be extracted and subsequently analyzed using PCR\(^{(6)}\).

Hypochlorite is a common component in household bleaches and cleaners, which are often used to remove blood from crime scenes. Cleaning agents not only have the potential to contaminate the biological material but may also degrade DNA present thus making the production of a conclusive and reliable profile difficult\(^{(7)}\), however, hypochlorite is volatile and comparably fast evaporates from a surface\(^{(8)}\). Harris et al. (2006)\(^{(9)}\) ran a multiphasic experiment. They washed various fabrics containing dried blood using various cleansers
SOMATIC SHORT TANDEM REPEAT (STR) DNA PROFILING OF HAND WASHED DETERGENT TREATED AND BLEACHED BLOOD STAINS

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Abdul Rauf Choudhry(****), Saif A. Al Rashidy(***)

Abstract

DNA evidence, linking perpetrators to crime scenes, is crucial to many legal proceedings. Blood is one of the most common physical evidence in investigations of violent crimes. Forensic analysis of the blood found at a crime scene supply in many ways valuable information that can be decisive in solving of a crime. Blood stains can be found anywhere a violent crime was committed.

Blood stain patterns on the floor (from a dripping wound, for example) or spattered on the walls can be interpreted for crime scene reconstruction. DNA typing analysis can establish the genetic profile(s) of the participant(s) in a violent crime. Consequently, blood stains are among the most useful evidence for court. This fact is becoming well-established so that criminals now often attempt to clean up their crime scene.

Blood samples were obtained from 5 unrelated donors and the substrates used in this study were pieces of white Saudi uniforms cloth (30% cotton and 70% silk), these pieces of cloth impregnate with blood were treated by different ways of hand washing including only water, soap compound (Arial), chlorinated bleach (Clorox), in addition soap and chlorinated bleach together.

DNA from blood stains were extracted and quantified. Autosomal STR genotyping of DNA isolated from hand-washed, detergent-treated and bleached blood stains were size separated on 3130 Genetic Analyzer.

Blood stain washed by water only were not affected. In the majority of samples complete autosomal STR profile were obtained from water or and from detergent washed blood stains whereas bleach-washing of the blood stains resulted in the loss of 2-3 autosomal STR loci. Blood stain washed with detergent and Clorox together, produced partial human DNA profile. Autosomal STR loci D7S820, CSFIPO and D18S51 appeared to the most be affected mostly by bleach and or detergent washing.

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