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# The effect of cleaning agents on the DNA analysis of blood stains deposited on different substrates

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**Abstract.** A deliberate attempt to remove biological material (using a variety of cleaning agents) is a problem faced by forensic scientists routinely. The substrates on which the blood is supported can also have an inhibitory role. This study aimed to investigate the potential contribution that these factors have on DNA analysis. In addition, time between deposition and collection of trace evidence was also considered. Blood samples were applied to a number of different substrates. After drying, the stains were cleaned with chlorinated bleach, soap or disinfectant until no visible trace remained. DNA was extracted from the cleaned areas and PCR performed using the AmpFLSTR® SGM Plus™ PCR Amplification kit (Applied Biosystems). In excess of 250 profiles were examined and characterised. Heterozygote imbalance (Hbx), split peak frequency (SPF) and stutter proportion (SP) were used to assess the clarity of the electropherograms and the ability to relate evidence and control suspect samples. In this 15-day study, chlorinated bleach had the most pronounced negative effects with respect to the characteristics considered. © 2006 Published by Elsevier B.V.

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## 1. Introduction

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 [1] and the sensitivity of the Kastle–Meyer (KM) presumptive blood

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test makes the sourcing of such trace evidence possible. In cases where blood stains have been deliberately removed by cleaning, visualisation of potential evidence is not the sole problem. 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. The nature of the support or substrate on which the blood is suspended can itself introduce contaminants to the evidential material. Dyes from fabrics or other water soluble compounds used in the textile industry can potentially inhibit PCR [2]. With trace evidence, the efficiency of the amplification process is reduced resulting in an increased occurrence of pronounced heterozygote imbalance, allelic dropout, stutter and non-specific artefacts. This study aimed to consider the extent to which different cleaning agents and support materials exaggerate these problems, hence making accurate profiling difficult.

## 2. Materials and methods

### *2.1. Samples, substrates and cleaning agents*

Blood samples were obtained from 6 unrelated donors. The substrates used in this study are shown in Table 1. Thick chlorinated bleach, general pine disinfectant and a non-chlorinated liquid detergent were used as cleaning agents.

### *2.2. Stain preparation and cleaning*

All substrates were UV irradiated for 20 min prior to stain deposition. A standard volume of blood was applied to each substrate before drying at room temperature. Cleaning agents were used according to manufacturer's instructions. Substrates were thoroughly cleaned until no visible trace of blood remained and then left to dry.

### *2.3. DNA extraction and detection*

The KM test was used to test for the presence of blood prior to extraction. DNA was extracted using Chelex®100, irrespective of KM result. For porous substrates, a 1 $cm^2$ section was used for extraction (in the case of carpet, individual fibres were used). For non-porous substrates a 2 $cm^2$ area was wet swabbed and the swab used for extraction. Duplicate extractions were carried out on days 1, 5, 11 and 15. PCR was performed using

Table 1
Substrates used as supporting medium

| Porous | Non-porous | Colour | Comments |
|---|---|---|---|
| – | Laminate flooring | – | Smooth, glossy surface |
| – | Vinyl flooring | – | Rough surface |
| – | Glass | – | – |
| Carpet | – | Various colours | Thick carpet |
| Fabric sample 1 | – | Beige | 100% cotton |
| Fabric sample 2 | – | Black | 100% cotton |
| Fabric sample 3 | – | Blue | 97% Cotton, 3% elastane (denim jeans) |
| Fabric sample 4 | – | Khaki | 55% Cotton, 45% polyester |
| Fabric sample 5 | – | White | 21% Cotton, 39% polyester, 40% rayon |
| Fabric sample 6 | – | Pink | 100% Wool |

the AmpFLSTR® SGM Plus™ PCR Amplification kit (Applied Biosystems). Products were detected using the ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems) and the denaturing polymer POP-6.

#### *2.3.1. 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 largest part in compromising the quality of the resultant profile.

### *2.4. Data analysis*

In excess of 250 profiles were examined and characterised using heterozygote imbalance (Hbx), split peak frequency (SPF) and stutter proportion (SP). For each known heterozygote locus, Hbx was calculated as follows: $\varphi_{min}/\varphi_{max}$ ($\varphi_{min}$=peak area of smallest peak, $\varphi_{max}$=peak area of largest peak). Locus specific SPF (total number of split peaks at a locus/total number of spilt peaks at all loci) and substrate specific SPF (total number of split peaks for a particular substrate/total number of split peaks for all substrates) were calculated. At each locus where a stutter peak was detected, SP was calculated as follows: area of stutter peak/area of allele peak. This information was used to assess the clarity of the electropherograms and the ability to relate evidence and control suspect samples, over a period of 15 days.

## 3. Results and discussion

Out of all the cleaning agents bleach had the most deleterious effect on the quality of the DNA profile obtained. In about 80% of cases, where a negative KM test was obtained, subsequent DNA extraction provided a profile from porous materials. Attempts to obtain profiles from non-porous materials were unreliable when duplicates were compared. In bleach treated materials the resultant profiles continued to decline in quality exhibiting increased scatter of Hbx over time, suggesting a continued degradation of the DNA. This was not seen in substrates cleaned with soap or non-chlorine disinfectant. Regardless of cleaning agent used, stutter peaks were observed with proportions less than 15%. Split peak frequencies were low (less than 16%) for all cleaning agents. Substrate type and time were found to be insignificant variables. DNA quality was found to be appreciably high despite the use of chlorinated and non-chlorinated cleaning agents.

## References

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