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Validation of forensic DNA analysis from bloodstains treated by presumptive test reagents

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Abstract. Dilute bloodstains that cannot be seen by the eye may be detected at the crime scene using different presumptive tests. In this study, we tested the effect of two presumptive blood enhancement reagents (luminol and fluorescein) on the subsequent chemical and genetic analysis of fresh or aged bloodstains treated or not treated by soap.

Keywords: Dilute bloodstains; Presumptive tests; Hemoglobin detection; STRs

1. Introduction

The enhancement reagents often provide valuable aid in crime scene analysis and laboratory examination: in fact, they are able to detect minute traces of blood even when attempts have been made to wash away the incriminating evidence. By the luminol test, the bloodstains can be made to glow with a blue light due to the chemiluminescent reaction of the luminol reagent with hemoglobin iron while fluorescin is converted by the catalytic action of hemoglobin into fluorescein, a bright-yellow fluorescent chemical. We evaluated the effect of the reagents above on the hemoglobin detection by spectrophotometry, chromatography and immunodiffusion; the ability to extract DNA using DNA IQ System (Promega), Chelex (Biorad) and phenol–chloroform treatment; any potential detrimental effect on STRs analysis using the Amp*FlSTR Profiler Plus* and *Identifiler* kits (Applied Biosystems); the possibility to obtain consistent and reliable DNA typing results.

2. Material and methods

We used an old blood sample to prepare some bloodstains (of about 3 × 3 cm) on the following surfaces: moquettes carpet, tissue, paper, wood, skin, glass, and tile. As a reference, we used a fresh blood sample belonging to the same donor above. All bloodstains were dried for about 1 week, and each one was used at natural and after

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washing with water and soap. Each surface was divided in two different fields, and each one was sprayed with luminol or fluorescein. From each area were taken three different samples.

Fluorescein and luminol preparation was performed, respectively, following the procedures described by Cheeseman et al. [1] and Eckert and James [2]. Detection limit using homemade reagents was 1:1,000,000. For immunodiffusion, we prepared a 3% agar solution following the protocol described by Hochmeister et al. [3]. DNA was extracted by DNA IQ System, Chelex, and by organic/centricon treatment.

As a reference, we extracted DNA from fresh and old blood samples, without any treatment. All samples were quantified by the Alu Quant Human DNA Quantitation System, then amplified by *AmpFlSTRs Identifiler* and *Profiler Plus* kits and PCR products analyzed onto two ABI PRISM 310 Genetic Analyzer.

3. Results and discussion

3.1. Spectrophotometry

To observe any eventual modification of hemoglobin typical spectra produced by reagents, each dilution was analysed (in the range 200–800 nm) by an HP spectrophotometer at 412 nm (Soret peak), 589–667 and 553–525 nm (Fraunhofer peaks). We determined also soap, luminol and fluorescein spectra. In the presence of soap, typical hemoglobin peaks were variably detectable with the kind of cleaning we performed, while in the presence of luminol and fluorescein, typical hemoglobin peaks become NOT REVEALABLE also on NOT WASHED bloodstains.

3.2. Chromatography

As confirmatory test for the presence of human hemoglobin, we use an immunochromatographic rapid test OC-Hemocard (Alfa Wassermann). The eluate obtained by distilled water from an untreated bloodstain showed pH 7 and gave a positive result with Hemocard test. The eluate obtained by distilled water from a bloodstain sprayed with luminol showed pH 11 and gave negative results with Hemocard test. The eluate obtained by distilled water from a bloodstain sprayed with fluorescein showed pH 8.5 and gave a weakly positive result. In both cases, adjusting pH to 7 by Tris–HCl or NaOH treatment before adding the sample to Hemocard, we obtained a positive result. When about 200 Al of IQ or phenol–cloroform extraction buffers were added to the Hemocard, a negative result was found.

Lysis buffers above require SDS, Proteinase K or DTT that interfere with the test presumably damaging hemoglobin. We found similar circumstances using Chelex sovranatant.

Results from washed and unwashed bloodstains were comparable: the possibility to obtain a positive result is dependent from the cleaning modality or intensity.

3.3. Immunodiffusion

We evaluated the possibility to detect hemoglobin on washed/unwashed bloodstains after reagent treatment using a commercially available anti-human hemoglobin antiserum.

Luminol and fluorescein do not interfere with the possibility to detect hemoglobin.

3.4. DNA analysis

We evaluated the ability to extract DNA using DNA IQ System, Chelex and organic/centricon procedure from bloodstains treated by luminol or fluorescein, any potential detrimental effect on STRs analysis and the possibility to obtain consistent and reliable DNA typing results. (a) Washed and unwashed samples treated by presumptive reagents were successfully quantified by the Alu Quant Human DNA Quantitation System. (b) Differences were noted on the ability to obtain DNA suitable for STRs analysis from the surfaces used in this study depending on the nature of the substrate. (c) The organic/centricon extraction method gave higher yields of DNA than Chelex or DNA IQ. (d) Profiles from fresh or old bloodstains treated were comparable to the ones of the untreated fresh or old blood samples. (e) Results obtained after luminol or fluorescein spraying were comparable (no evidence of degradation correlated to the reagents applications). (f) Differences were noted between DNA profile of old blood samples (partial profile, allelic dropout, unbalanced peaks) and the ones of fresh samples (full profile). (g) Soap does not interfere with the ability to recover DNA: the quality of DNA profile is dependent from the washing modality or intensity.

4. Conclusion

Luminol or fluorescein enhancement of bloodstains interferes with the possibility to perform spectrophotometrical determination of hemoglobin, probably because the absorption peaks of reagents above cover the hemoglobin peaks and prevent from discovering them. Both reagents do not interfere with the confirmatory tests that use antihuman hemoglobin monoclonal antibody: it is very important to adjust pH to values around 7 so as to obtain a good test working.

Reagents do not interfere with the ability to obtain DNA suitable for PCR analysis: the substrate, the cleaning, the age of the sample and the choice of the DNA extraction method are the major factors that can affect the success of DNA analysis. Results obtained from washed and unwashed bloodstains were comparable: a positive result can be dependent from the washing modality or intensity.

Our results are in accordance with studies previously published by other [4–6].

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