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A comparative study of the sensitivity and specifity of luminal and fluorescein on diluted and aged bloodstains and subsequent STRs typing

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Abstract. Luminol and fluorescein are very important reagents for diluted and aged Bloodstain detection at crime scene. The aim of this study was to carry out a comparative study of the sensitive and selectivity of these two presumptive blood tests using a series of diluted blood (from 1:10 to 1:10.000.000) on a large variety of substrates, as well as, to evaluate the ability to type STRs on treated samples. © 2006 Published by Elsevier B.V.

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1. Introduction

Presumptive reagents, such as luminol and fluorescein, are largely used to detect dilute blood that can not be seen by eye. Although their utility is undoubted, in deciding the optimal reagent to use is desirable to appreciate their utility and limitations, considering false positive reactions, dilution of traces, sensibility on extremely diluted trace and last but not least, limitations for DNA typing, using the most recent STRs markers commercially available.

2. Materials and methods

Blood from a single donor, drawn by venipuncture into an EDTA tube was diluted 1:10, 1:1.000, 1:100.000, 1:10.000.000 with physiological buffer. These four groups of solutions were then called Co1, Co2, Co3 and Co4. One hundred ul aliquots of diluted blood were

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then deposited on nine different substrates (labelled from A to I, Table 1), and allowed to dry and age for 3 weeks. Luminol and fluorescein (in the reduced state) were prepared fresh according to Budowle et al. [1,2] and subsequently applied on the entire surface of the stains. Luminol-treated stains were viewed in complete dark with naked eye, while fluorescein stained materials were viewed using an alternate light source (Crimescope CS16 Spex Yobin-Jvon), set at 450 nm. Bloodstains were collected both by cutting or by swabbing with "OralSwab" (Whatman) wetted with distilled water. DNA extraction was performed with the commercial kit "ChargheSwitch Forensic DNA Purification Kit" (Invitrogen) according to the manufacturer's protocol. Genotyping was carried out by using AmpF*l*STR® Identifiler™ DNA Amplification Kit from Applied Biosystems and PowerPlex 16.2 kit (Promega) according to the protocol provided by the suppliers. Results were analysed with GeneMapper v.3.2 (ABD). Due to the results obtained by DNA quantification, PCR amplification was limited only to the dilutions set Co1 and Co2.

3. Results and discussion

The detection ability of both reagents is shown in Table 1.
Results of genetic analyses are shown in Tables 2 and 3.

Table 1
+ detectable, − not detectable

	Luminol				Florescein			
	Co1	Co2	Co3	Co4	Co1	Co2	Co3	Co4
Wood	+	+	+	−	+	+	−	−
Stone	+	+	+	+	+	+	+	+
Iron	+	+	−	−	+	+	+	+
Plastic	+	+	+	+	+	+	+	+
Rubber	+	+	+	−	+	+	−	−
Wax-cloth	+	+	+	+	+	+	+	+
Baked clay	+	+	+	−	+	+	+	+
Linoleum	+	+	+	+	+	+	+	+
Cotton	+	+	−	−	+	+	−	−

Table 2
DNA quantification and profile quality (n.d., not detected, *stochastic effects) of sample from stain Co1 diluted

Dilution 1:10 (Co 1)							
Sample	Substrate	Real-time PCR (ηg/μl)		Quantiblot (ηg/μl)		RFU of the DNA profiles	
		LUM	FLU	LUM	FLU	LUM	FLU
1A	Wood	0.019	0.024	0.052	n.d.	0–400*	0–300*
1B	Stone	0.440	1.106	n.d.	0.247	500–5000	300–2500
1C	Iron	0.339	0.472	n.d.	0.070	200–2000*	2000–5000
1D	Plastic	3.774	2.332	0.634	0.463	3000–8000	3000–7000
1E	Rubber	0.608	1.880	0.153	0.531	2000–6000	3000–7000
1F	Wax-cloth	2.099	1.190	0.279	0.253	1000–5000	2000–6000
1G	Baked clay	2.727	1.023	0.256	0.286	2000–6000	800–3000
1H	Linoleum	1.390	0.447	0.228	0.081	2000–6000	4000–9000
1I	Cotton	1.031	0.634	0.063	0.660	2000–7000	500–3000

Table 3
DNA quantification and profile quality (n.d., not detected, *stochastic effects) of sample from stain Co2 diluted

Dilution 1:1.000 (Co 2)							
Sample	Substrate	Real-time PCR (ηg/μl)		Quantiblot (ηg/μl)		RFU of the DNA profiles	
		LUM	FLU	LUM	FLU	LUM	FLU
2A	Wood	0.013	0.003	n.d.	n.d.	100–600*	100–600*
2B	Stone	0.006	0.040	n.d.	n.d.	100–300*	200–800*
2C	Iron	n.d.	n.d.	0.043	n.d.	0–500*	0–250*
2D	Plastic	0.179	0.087	0.048	n.d.	200–1500	200–2000*
2E	Rubber	0.126	0.088	n.d.	0.056	400–2000	500–2000*
2F	Wax-cloth	0.107	0.198	n.d.	0.051	100–2000*	1000–4000
2G	Baked clay	0.082	0.059	0.081	n.d.	100–500*	100–1000*
2H	Linoleum	0.153	0.035	0.034	n.d.	100–500*	100–600*
2I	Cotton	0.050	0.056	0.064	0.036	100–300*	300–1500

As expected, luminol and fluorescein have shown different detection capabilities depending upon the substrate. Luminol compared to fluorescein seems to be more sensitive on wood and rubber (Co3 instead of Co2 dilution) while fluorescein has shown more sensitivity on iron and baked-clay (Co4 respectively instead of Co2 and Co3). As it regards stone, iron, plastic and wax-cloth, both reagents have shown the same huge sensitivity, till the last dilution Co4. Cotton, instead, gave different results, as we were able to detect bloodstains just until Co2. Our results confirm that non-absorbent substrates offer a better surface to detect blood, independently of the reagents used as showed by Budowle et al. [1]. Relevant DNA typing has demonstrated that it is possible to have a blood dilution up to 1:1000, corresponding to a DNA quantity of about 50 pg/μl, independently of the reagent used to treat the sample, showing results never seen in previous studies. Even if no statistical tests have been conducted, no significant difference, regarding the DNA extraction as well as the capability to be amplified, can be deducted from the dataset. Independently of the presumptive reagent used, it was not possible to obtain typeable results from extremely diluted bloodstains (Co3 and Co4, data not shown). Stochastic effects, such as drop-out or missing genotype have occurred during the PCR of Co2 diluted samples, due to the very low quantity of DNA rather than being affected by the luminol or the fluorescein treatment. In conclusion, the results of the current study confirm that either luminol or fluorescein are excellent reagents to detect latent and aged bloodstain at crime scene. They also showed that their use does not interfere with the ability to recover typeable DNA. Using the most recent STRs markers, it was possible to obtain full DNA profiles even at very high blood dilutions (i.e. 1:1000), obtaining results never achieved in the past, which encourage their use for criminal investigation.

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