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DNA profiling by different extraction methods

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Abstract. The aim of this study is to compare the efficiency of different validated methods for DNA extraction using commercial kits. DNA extraction was performed on fresh liquid blood, old bloodstains, cigarettes butts, semen stains and hairs. Samples were quantified by the AluQuant Human DNA Quantitation System. Amplification was carried out by GeneAmp 2400 and 9700 Thermal Cyclers using the AmpFlSTR Identifiler kit. Amplified products were analyzed into both ABI PRISM 310 and 3100 Genetic Analyzers employing ABI softwares.

Keywords: Extraction methods; Forensic samples; STRs

1. Introduction

In recent years a number of different forensic kits for recovering small amounts of DNA from a variety of specimens (saliva, sperm stain, clotted blood) has been developed in order to improve the final quantity of DNA recovered, thus substantially reducing the cost per analysis.

The aim of this study was to evaluate the ability of each method to extract DNA from different samples; the quantity of human DNA extracted from each sample; any potential interference due to the substrate; the possibility to obtain reliable DNA typing results; and the quality of profiles obtained.

The present paper is intended to be the first step of an exhaustive research that will be soon extended to more systems, therefore here we will report our preliminary results and evaluations.

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2. Material and methods

We used 16 bloodstains (from 4 to 1 years old) upon cotton, denim, wood; 10 cigarette butts (6 months old); 10 anagen and 14 telogen single hair with roots; 10 semen stains on paper and tissue (3 months old).

For referencing, we spotted 30 μl of fresh blood on surfaces like cotton, denim, wood.

We took a piece of bloodstains and semen-stains in a known quantity with Harris UNI-CORETM cutter 2.0 mm.

DNA was extracted with DNA IQTM Systems (Promega), Invisorb Forensik Kit I (Invitek), Chelex (Biorad), QIAamp DNA Blood Mini kit (Quiagen, Hilden, Germany) and phenol–chloroform treatment according to the manufacture's protocol. [1–6] Quantification of human DNA was provided by the AluQuant Human DNA Quantitation System (Promega), following the manufacture's protocol [7].

Amplification was carried out in a different laboratory from the one dedicated to the extraction, so that amplified products never entered the extraction laboratory.

STRs amplification was carried out by GeneAmp 9700 and 2400 thermal cyclers, by the AmpFLSTR Identifiler kit (Applied Biosystem), according to the kit protocol, positive and negative controls were enclosed during the amplification step [8].

Amplified products were analyzed by capillary electrophoresis into both ABI PRISM 310 and 3100 Genetic Analyzers employing ABI softwares (DATA Collection, GeneScan Analysis, Genotyper Fragment Analysis).

3. Results and discussion

Our evaluations are summarized as follows: (a) the commercialized protocols should be adapted to improve the sensitivity when working either with difficult samples (old blood or old bloodstains on surfaces like wood or denim) or samples containing small DNA amount (telogen single hairs); (b) all the five procedure performed gave good and reproducible results in presence of fresh bloodstains. (c) the system based on colon like QIAamp kit constitutes an excellent method of purification, mainly for traces containing inhibitors; nevertheless, we observed a loss in DNA recovering (around 30%) that however is dependent from different factors (quantity of eluition buffer, no. of eluitions, eluition time and temperature). (d) for blood traces on denim and on nude wood (inhibitors presence) the QIAamp system gave the best results in comparison with the other methods, being able to purify completely the sample; however, the success of the analysis is also highly dependent on the modality of sampling (blood eluition or scraping). The kind of denim fiber and its color (clear or dark) are very relevant for the success of DNA extraction, since different tissues differently release DNA inhibitors. (e) systems like IQ allow a better surrender and an excellent reproducibility since the paramagnetic IQ resin used binds a known quantity of DNA and there is a low number of variable factors; the use of a single tube for DNA extraction reduces the loss of material. The process of lysis is excellent, thanks to an excellent buffer because of it the method is optimal for hard samples like single hair and semen stains too; we obtained a positive result for all 10 anagen single hair and for 9 of the 14 telogen hairs analyzed. Such a strong lysis solution may however interfere with the extraction of samples like cigarette butts since a prolonged exposition to it can destroy the filter that may link aspecifically the chelating resin. However, the strong IQ lysis solution

(that contains DTT and PK), if efficiently opens sperm cells, nevertheless, may destroy the stain substrate (especially paper) and produce interferences that reduce the extraction output. (f) Chelex represents the optimal method when referred to low cost and rapidity of execution in a single tube; therefore its use is suggested with common samples as cigarette butts, semen stains, bloodstains. However, a single step does not remove DNA inhibitors that could interfere with DNA typing and so in their presence, a passage in centricon or microcon could be required to purify the sample. A successful DNA extraction of blood traces on jeans and nude wood has been observed using Chelex only when the blood sampling was performed by eluition of the stain from the surface. A similar situation was observed employing DNA IQ or Invisorb procedures. The main problem using chelex for old bloodstains is to make the stain soluble: since the reagent is not very strong, the procedure may require a prolonged incubation time to eluite the trace. IQ treatment permits to solubilize better the old stain since it uses a very strong extraction buffer. Semen stains gave similar good results using IQ or Chelex procedures. (g) Phenol–chloroform procedure employs many dangerous reagents, it is time-consuming since it requires many steps and a particular accuracy to avoid the loss of material, especially when they are analyzed samples containing LCN such as cigarette butts, hairs or semen stains. Organic extraction is restricted to a few complex cases only when samples are very degraded (old washed bloodstains, bones, putrefied tissues) since this procedure plus Centricon concentration, in comparison with other ones, permits to obtain a better DNA extract. (h) Invisorb procedure uses a soft buffer so we did not observe optimal results using it for extracting old bloodstains or bloodstains on wood or denim. However, it can be used with excellent results for cigarette butts and fresh blood.

4. Conclusion

A forensic laboratory often has to deal with samples that are less than ideal since the evidence may have been left exposed to hard environment for a long time or can be found on varied surfaces. In fact, a biological sample found on a crime scene as a liquid blood sample or more often as a bloodstain or semen stain or hair can be degraded or contains a number of substance besides DNA that can inhibit PCR amplification.

For example, materials like textile dyes or hemoglobin can remain with the DNA throughout the sample preparation process and compromise successful PCR.

Our paper shows that a right choice of the DNA extraction method and an accurate DNA quantification are very important steps in the analytical procedure to ensure optimal results.

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