

# DNA typing from 15-year-old bloodstains

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**Abstract.** The aim of this study is to compare the efficiency of different validated methods for DNA extraction on old bloodstains. The study has been performed on bloodstains placed on a cotton surface, stored at room temperature for 15 years. We have evaluated the ability of each method to extract DNA, the quantity of human DNA extracted with each procedure, the ability to perform multiplex STRs amplification and the reproducibility of results obtained. © 2006 Published by Elsevier B.V.

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## 1. Introduction

The aim of this study is to compare the efficiency of different validated methods for DNA extraction on old bloodstains. The study has been performed on bloodstains placed on a cotton surface, stored at room temperature for 15 years.

## 2. Materials and methods

The study has been performed on bloodstains placed on a cotton surface, stored at room temperature for 15 years. As reference were used liquid blood samples, stored at  $-20^{\circ}\text{C}$ , belonging to the same donor. DNA has been extracted from bloodstain samples using different procedures: Chelex (Instant Gene Matrix, Biorad), paramagnetic silica particles (IQSystems, Promega), silica membrane column (NucleoSpin, Macherey Nagel), and desalting procedure (MasterPure, Epicentre) [1–5].

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For each extraction procedure, we used three 3-mm punches removed from the sample area by a special cutter.

In order to minimize the possibility of contamination, all extractions were set-up in a Gelman laminar flow cabinet in a dedicated laboratory.

DNA was extracted, by “Instant Gene Matrix” (Biorad) treatment, from the liquid blood sample used as reference belonging to a healthy donor. DNA extracted from samples above were quantified by the Quantifiler™ Human DNA Quantification Kit using a 7300 Real Time System kit (Applied Biosystems) following the manufacturer protocol.

Amplification was carried out in a laboratory different from the one dedicated to the extraction so that amplified products never entered the extraction laboratory. STR amplification was carried out according to the AmpF/STR Identifiler (Applied Biosystems) protocol using the GeneAmp PCR Systems 9700,2720 thermal cyclers (Applied Biosystems) [6].

Positive and negative controls were used during all amplification steps.

The cycling reactions were started with an initial 94-s delay for 12 min and the number of PCR cycles increased until it reached 35.

Amplified samples were then analyzed on an ABI PRISM 3130 multicapillary sequencer employing GeneMapper v.3.2 software.

For fragment length determination of the products, the internal lane DNA standard LIZ 500 (Applied Biosystems) was used for calibration.

### **3. Results and discussion**

In the present study have been evaluated: the ability of each method to extract DNA, the quantity of human DNA extracted with each procedure, the ability to perform multiplex STRs amplification, the possibility to obtain reliable DNA typing results. The quality of DNA profiles obtained by the above extraction procedures (presence of partial profile, unbalanced peaks, or allelic drop out).

On the basis of the above factors, we have observed that:

- a) Systems based on silica membrane columns like NucleoSpin kit constitutes an excellent and rapid method of purification. It gave the best results in comparison with the other methods being able to elute totally the stain and to purify completely the sample. In fact, we obtained full DNA profiles, with balanced peaks, low noise and high reproducibility.
- b) Systems like IQ allow good reproducibility, as the paramagnetic IQ resin used, binds a known quantity of DNA and, besides the use of a single tube for DNA extraction, reduces the loss of material. The strong lysis solution may be useful for removing efficiently the stain from the substrate without destroying it, but not all inhibitors are removed and not satisfying profiles are often found.
- c) Chelex represents the optimal method when referred to low cost and rapidity of execution in a single tube. The main problem using Chelex for DNA extraction from old blood traces is to make the stain soluble: since the reagent is not very strong, the procedure requires a prolonged incubation time to elute the trace. In fact, DNA profiles obtained following the traditional extraction procedure showed only few loci, while by

adding an aliquot of proteinase K about 10 mg/ml and with a prolonged incubation time, better profiles were obtained. Since a Chelex single step does not remove DNA inhibitors that could interfere with DNA typing, a passage in columns (as centricon or microcon) is required to purify the sample.

- d) Desalting procedure like MasterPure treatment is fast and permits to solubilize excellently the old stain since it uses a very strong extraction buffer. But depending on the nature of substrate, adjustments in nuclease concentration or time of incubation may be useful for improving the quantity of extracted DNA. The quality of DNA profiles obtained is quite good, with balanced peaks.

#### 4. Conclusions

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 in the form of a liquid blood sample or more often as a bloodstain can be degraded or may contain a number of substance besides DNA that can inhibit PCR amplification.

Commercialized protocols should be adapted to improve the sensitivity when working either with difficult samples such as old bloodstains. For example, materials such as hemoglobin can remain with the DNA throughout the sample preparation process and compromise successful PCR.

Our paper show that a right choice of the DNA extraction method and an accurate DNA quantification are very critical steps and that a right analytical procedure permits to obtain good results even when we are working with old samples such as 15-year-old bloodstains.

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