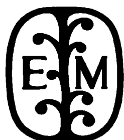

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# DNA typing from biological stains: a casework experience

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**Abstract**

This study presents the results of DNA analysis obtained from biological stains and particularly non-haematological stains and its direct application to forensic casework. DNA was extracted using two different methods (Chelex and pCIA) and analysis performed by manual procedures (Dot-Blot analysis-silver staining with denaturating electrophoresis) and by automated procedures (AmpFlSTR Profiler Plus and SGM with an ABI 310).
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*Keywords:* Biological stains; DNA typing; Human identification

## 1. Introduction

In a crime scene many different exhibits can be found and a forensic lab can be asked to examine the biological stains identified upon them. By an analysis of the literature, many publications have reported analysis carried out on blood stains, sperm, vaginal swabs, saliva or biological mixtures, while more rarely other studies have examined urine, faeces or nasal mucus, perspiration and tears. The aim of this study was to optimize PCR DNA typing of non-haematological fluid with direct application to forensic casework.

## 2. Materials and methods

### *2.1. Samples*

DNA was obtained from experimental stains created to simulate true cases and from sequestrated exhibits with different procedure.

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Experimental stains were prepared, according to the literature, as follows: some body fluids were directly taken from the donor and absorbed on cotton cloth, paper towels or cotton wool tips, air dried and stored at room temperature for 1 to 30 days prior to analysis. Afterwards, they were cut into small pieces and utilised for the analysis [1–3].

Stains from exhibits were cut and used directly; for the saliva stains on cigarettes, a fragment of the end of the cigarette paper was used [4].

## 2.2. DNA extraction

DNA was extracted using two different procedures: Chelex and the standard procedure with pCIA after the digestion with proteinase K followed in some cases by purification with Quiamp (Quiagen).

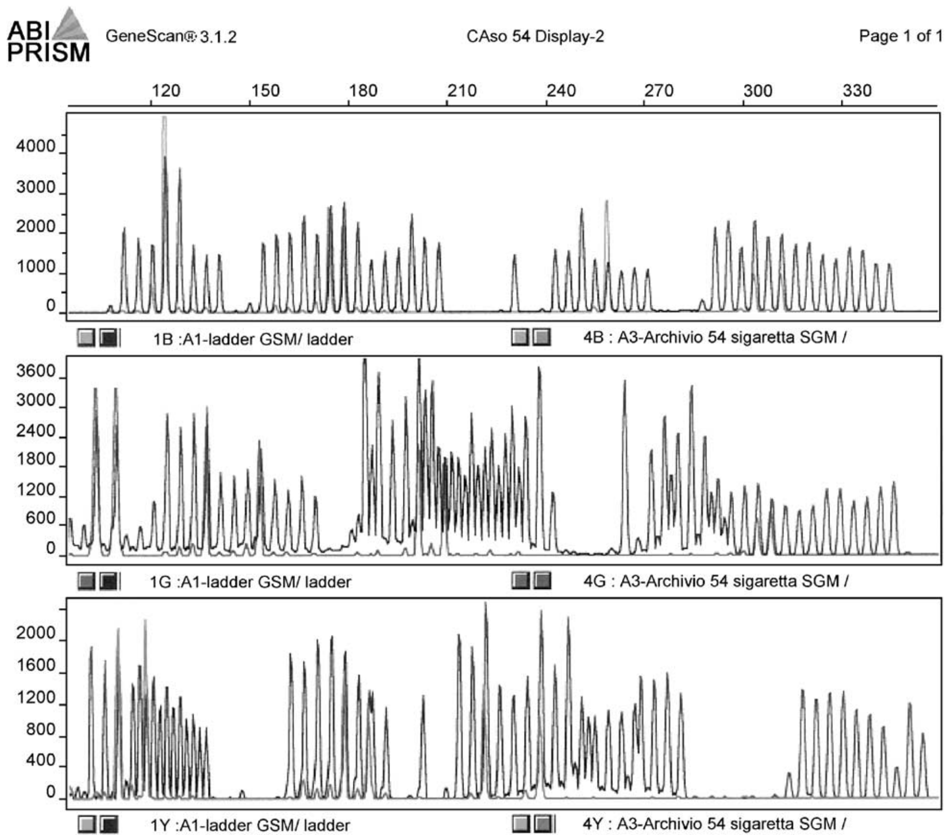


Fig. 1. Gene Scan electropherogram of AmpFlSTR SGM alleles. In this example DNA is extracted from cigarette end butt.

### 2.3. Amplification and detection

DNA was amplified and analysed at first with manual procedures and subsequently by automated procedures as follows.

(1) Dot Blot analysis: HLA-DQα and Polymarker. Amplitype PM+, DQA1 forensic amplication and typing kit (Perkin Elmer) performed according to the kit protocol.

(2) STR multiplex or singleplex GenePrint STR Systems from Promega (CSF1PO, TPOX, TH01, F13A01, F13B, FESFPF, VWF, LPL and Amelogenin); performed according to the kit's protocol; PCR products were run in 0.8 mm vertical 6% denaturing polyacrylamide gels followed by silver staining.

(3) Ampli FlSTR$_s$ from Applied Biosystem Profiler Plus and SGM kit analysed by capillary electrophoresis on an ABI 310 instrument. Fragment size and genotype were determined with Genescan and Genotyper software (Fig. 1).

## 3. Results and discussion

In the first case, good results with the Chelex extraction protocol were obtained with Ampli FlSTR studies performed using the automated procedure and in some cases for DQ-A1 and Polymarker. Poorer results were obtained from the application of the Chelex protocol with STR analysis performed by manual methods; for this protocol better results were obtained with phenol chloroform extraction.

DNA extracted from vaginal swab, post-coital samples and sperm was successfully used for all the three different analytical procedures (Dot Blot, Manual electrophoresis and Automatic separation) and in two cases of post-coital sample analysis, mixed DNA profiles were identified even with a negative microscopic result.

Successful results were obtained from nasal mucus, saliva samples and particularly from cigarette ends using automated procedures: for manual procedure, the DNA extracted was purified and concentrated. From urine stains and faecal samples, we obtained results only with automated procedures, being more sensitive, since there is much less DNA and more PCR inhibitors present. In the same way, amplification was unsuccessful with perspiration samples while DNA extracted from tears has supplied clear profiles only by using automatic procedure and DQα and PM kit.

The positive results obtained in this study confirmed what is reported in the literature and encourage us to further the application to simulate true cases waiting for a real concrete application in forensic casework.

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