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Research article

Study about the effect of high temperatures on STRs typing

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Abstract

Forensic investigations generally involve samples that have unknown storage conditions.

These conditions may help to speed up or slow down the degradation of DNA. For example environmental factors that speed up the decay include: UV light, humidity, and temperature. The aim of the present study is to evaluate the effect of high temperature on the ability to perform DNA extraction and typing from different biological fluids (blood, saliva, and semen) after 20 min incubation in an oven at different temperatures 50 °C, 100 °C, 150 °C, and 200 °C or direct exposition to the effect of a flame for few minutes. Our results support the ability to type DNA even from samples exposed to drastic condition.
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Keywords: Temperature; DNA degradation; STRs

1. Introduction

Forensic investigations generally involve samples that have unknown storage conditions.

These conditions may help to speed up or slow down the degradation of DNA. For example environmental factors that speed up the decay include: UV light, humidity, and temperature. The humidity has more of an effect on the quality of the DNA, rather than the quantity. Low temperature seems to have a preservation effect on the DNA, while high temperature shows a significant loss in DNA [1].

The aim of the present study is to evaluate the effect of high temperature on the ability to perform DNA extraction and typing from different biological fluids (blood, saliva, and semen).

2. Materials and methods

2.1. Sample preparation

From each biological fluid (blood, saliva, and semen) were prepared 6 slides, using the same quantity of sample from a known male donor. Around 50 μl of each sample were deposited and smeared onto different slides that were left dried at room temperature for 2 days. Slides were treated as follows:

- 4 slides were incubated for 20 min in an oven at different temperatures 50 °C, 100 °C, 150 °C, and 200 °C;
- 1 slide was directly exposed to the effect of a flame for few minutes;
- 1 slide without any treatment, was used as reference.

Slides were stored for 24 h at room temperature.

Biological material was collected swabbing gently each slide by 2 different sterile swabs moist with distilled water. All swabs were then stored at 2–8 °C till the DNA extraction procedure.

2.2. DNA extraction and amplification

In order to minimize the possibility of contamination, all extractions were set up in a laminar flow cabinet in a dedicated laboratory. Per each slide, one swab has been extracted by *Instant Gene Matrix* (Biorad) and the other one by *Nucleo Spin* treatment (Macherey Nagel) [2–4].

For Chelex procedure, swabs were directly incubated at 56 °C for 2 h in about 200 μl of "*Instant Gene Matrix*" containing 5 μl of proteinase K 10 mg/ml and then boiled for 8 min.

DNA extracted was then purified and concentrated by ultrafiltration in microcon 100.

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Table 1
Evaluation scale of blood samples typing

	Instant Gene Matrix Identifiler	Instant Gene Matrix Nonaplex II	Nucleospin Identifiler	Nucleospin Nonaplex II
50 °C	++++	++++	++++	++++
100 °C	++++(+++)	++++	++++	++++
150 °C	+++	+++	+++	+++(++++)
200 °C	++	++	++	+++
Flame	+++	+++	+++	+++(++++)

++: sufficient amplification, +++: good amplification, ++++: excellent amplification.

For NucleoSpin procedure swabs were incubated in lysis buffer containing proteinase K at 70 °C for 15 min. Two wash steps were then done in the NucleoSpin® columns following the protocol. Samples were than eluted in 100 μl of prewarmed elution buffer after 10 min incubation.

All samples were then quantified in Real-Time PCR by the Quantifiler Y Male Human DNA Quantification kit using a 7300 Real-Time PCR System [5].

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

DNA was amplified either by A*mpFlSTR Identifiler kit* (Applied Biosystems) that amplifies simultaneously 15 STRs loci (D19S433, D3S1358, D5S8118, D8S1179, vWA, TH01, D13S317, D21S11, TPOX, FGA, D7S820, D16S539, D18S51, CSF1PO, D2S1338 and Amelogenin) than by *Nonaplex II PCR Amplification kit* (Mentype®) that amplifies simultaneously 8 polymorphic STRs loci (D3S1358, D8S1179, D18S51, D21S11, FGA (FIBRA), SE33 (ACTBP8), TH01 (TC11) and vWA as well as the gender-specific Amelogenin), following the manufacturer protocol [6,7]. Different positive and negative controls were enclosed during the amplification steps. Amplified samples have been analyzed on an ABI PRISM 3130 multicapillary sequencer employing GeneMapper 3.2 software.

3. Results and discussion

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 that may speed up the degradation of DNA.

It was compared the efficiency of 2 different validated methods for their ability to purify amplifiable DNA from samples in drastic conditions. Chelex represents the optimal method when referred to low cost and rapidity of execution in a single tube; however a Chelex single step does not remove DNA contaminants that could interfere with DNA typing and so in presence of them a passage in columns (as centricon or microcon) is generally required to purify the sample. DNA extraction procedure employing silica membrane column (NS Tissue kit) gave good results showing a high extraction power and an efficient purification of genomic DNA. However in both cases during Real-Time quantitation it was not observed inhibition of the internal control (IPC). The ability to quantify the amount of DNA extracted is critical in order to obtain a reliable DNA profile (Tables 1 and 2). Data from quantification show that high temperature produces in biological samples loss in DNA. In fact it has been observed that while there are not significative differences between not treated samples and samples after a 50 °C or 100 °C exposure, variation of temperature, from 100 °C to 150 °C determines around 50%

Table 2
Evaluation scale of saliva samples typing

	Instant Gene Matrix Identifiler	Instant Gene Matrix Nonaplex II	Nucleospin Identifiler	Nucleospin Nonaplex II
50 °C	++++	++++	++++	++++
100 °C	+++	+++(++++)	+++	+++(++++)
150 °C	++(+++)	+++(++)	+++(++)	+++
200 °C	+	+(++)	+	++(+)
Flame	++(+++)	+++(++)	+++(++)	+++

+: poor amplification (partial profiles), ++: sufficient amplification, +++: good amplification, ++++: excellent amplification.

Table 3
Evaluation scale of semen samples typing

	Instant Gene Matrix Identifiler	Instant Gene Matrix Nonaplex II	Nucleospin Identifiler	Nucleospin Nonaplex II
50 °C	++++	++++	++++	++++
100 °C	+++(++++)	++++(+++)	++++(+++)	++++
150 °C	+++(++)	+++	+++	+++
200 °C	+(++)	++(+)	++(+)	++
Flame	+++(++)	+++	+++	+++

+: poor amplification (partial profiles), ++: sufficient amplification, +++: good amplification, ++++: excellent amplification.

reduction of the initial DNA concentration independent of the sample nature, while from 150°C to 200 °C there is a 75% reduction of previous DNA concentration.

Sample exposition to a direct flame for few minutes produces a similar effect than incubation for 20 min in an oven at 150 °C. Anyway in all cases the amount of DNA was more than 100 pg and so enough for performing ordinary PCR. Either common STRs markers from Identifiler kit than mini STRs from Mentype® Nonaplex II PCR were analyzed. Common short tandem repeat (STR) markers are capable of generating typing results from very degraded material but the reduction in size of these amplicons can facilitate the examination and analysis of degraded DNA evidence by improving amplification efficiency.

In fact the quality of DNA profiles obtained using both kits and in particular the presence of partial profiles, unbalanced peaks, allelic drop out, the reproducibility of results were evaluated and compared. An evaluation 4 degree scale has been settled by means of a cross comparison between different methods, referring to both quantity of DNA extracts and quality of DNA profiles (see Tables 1–3).

At 50–150 °C all samples showed full DNA profiles independently from their nature and the modality of DNA extraction and amplification. With increased temperatures were found variable situations: Identifiler kit produced full profiles with blood and in the other cases partial profiles with allelic or locus dropout, while experiments performed with mini STRs generally gave better profiles showing less noise, even if due to the high degradation degree an imbalance could be observed between the smaller and bigger sized loci or between the smaller and the bigger sized alleles at the same locus in a heterozygote.

However with mini STRs was increased the chance to obtain full profiles because PCR products are reduced in size when compared to standard STR kits that generally generate amplicons in the size range of 100–450 s [8]. In general it was possible to identify clearly alleles since DNA profiles obtained were reproducible in all PCR tests performed on all biological samples even if it was observed that blood samples gave better results.

4. Conclusion

Forensic investigations generally involve samples that have unknown storage conditions.

These conditions may help to speed up or slow down the degradation of DNA.

Our data show that it is possible to type with success biological fluids as blood, saliva, and semen exposed to drastic conditions, even if high temperature produces a significant loss in DNA.

The chance of obtaining good results from a degraded sample is increased when mini STRs are analyzed because of the small sizes of STRs that are amplified.

Conflict of interest

None.

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