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The effect of freezing, thawing and long-term storage on forensic DNA extracts

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ABSTRACT

Following forensic DNA profiling (extraction, quantification and STR typing) the remaining extract is generally stored frozen. Our routine at the Swedish National Forensic Centre is to immediately after analysis freeze the sample. If a subsequent reanalysis is needed the sample is thawed and then refrozen. In this study the effects of freezing and thawing as well as long-term storage of DNA extracts in refrigerator or freezer have been investigated. The following sample types were extracted: two levels of blood and saliva, saliva on cigarette filter paper, saliva on cotton swabs and a combination of saliva and semen to mimic samples from sexual assaults. All extraction methods used were Chelex-based, DNA quantification was performed using PowerQuant System and STR profiling with PowerPlex ESX 16 Fast System. The study was divided into three parts: 1) freezing and thawing the extracts up to ten times, 2) storage in refrigerator or freezer up to four weeks and 3) long-term storage in refrigerator or freezer for 3, 6, 9, 12 and 35 months. Generally, the quantification and STR typing results show no indication of degradation after repeated freezing and thawing or long-term storage in refrigerator or freezer.

1. Introduction

Following forensic DNA profiling the remaining extract is generally stored in refrigerator (short-term) or freezer (long-term). If a subsequent reanalysis is needed the sample is thawed and then refrozen. In this study the effects of freezing and thawing as well as long-term storage of DNA extracts in refrigerator or freezer have been investigated.

2. Materials and methods

The following sample types were extracted: two levels of blood and saliva, saliva on cigarette filter paper, saliva on cotton swabs and a combination of saliva and semen to mimic samples from sexual assaults (epithelial and sperm). DNA from the epithelial and sperm samples was extracted using a Chelex-based differential extraction method where the final step include purification using a filter device (Amicon Ultra-2 30K, Merck Millipore). For the other samples a Chelex-based one-tube direct lysis DNA extraction protocol was used [1,2]. DNA quantification was performed using PowerQuant System (Promega Corporation) on Applied Biosystems 7500 Real-Time PCR System (Thermo Fisher Scientific) and STR analysis was performed applying PowerPlex ESX 16 Fast System (Promega Corporation), Veriti 96-Well Thermal Cycler, Applied Biosystems 3500xL Genetic Analyzer, and GeneMapper ID-X Software v1.6 (Thermo Fisher Scientific). The study was divided into three parts:

1) Freezing and thawing the extracts up to ten times. Triplicates were extracted and every time a sample was thawed it was immediately quantified. STR profiling was performed twice, every fifth freezing/thawing cycle.
2) Storage in refrigerator or freezer up to four weeks. Triplicates were extracted and for samples stored in the freezer new samples were thawed every week while samples kept in the refrigerator were the same samples for the whole period. Samples were quantified weekly and STR profiling were performed every other week.
3) Long-term storage in refrigerator or freezer where the samples were reanalysed after 3, 6, 9, 12 and 35 months. Triplicates were extracted and for samples stored in the freezer new samples were thawed every occasion while for samples kept in the refrigerator the same samples were used for the whole period.

DNA concentration, qPCR degradation index ([Autosomal]/

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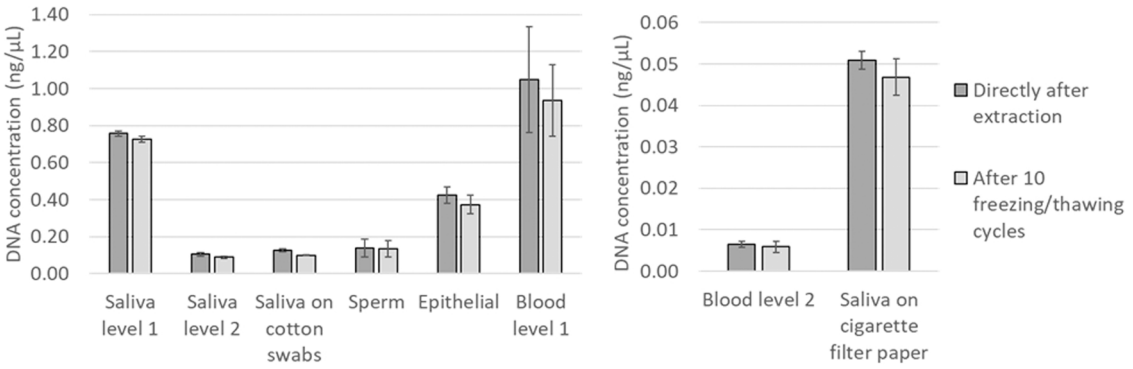

Fig. 1. Effect of freezing and thawing of DNA extracts. DNA concentrations (ng/µL) measured directly after DNA extraction (dark grey) and after ten freezing and thawing cycles (light grey) (n = 3). Mean values and standard deviations are presented. N.B. the different scales on the y-axes.

Table 1
Effect of long-term storage of DNA extracts. DNA concentrations (ng/µL) measured directly after extraction (reference) and following storage during 6, 12 and 35 months in refrigerator (R) or freezer (F) (n = 3). For a few samples the liquid had evaporated after 35 months so no replicates were analysed (N/A: not available). Mean values and standard deviations are presented.

Storage	Saliva level 1	Saliva level 2	Saliva on cotton swabs	Sperm	Epithelial	Blood level 1	Blood level 2	Saliva on cigarette filter paper
Reference	0.71 ± 0.07	0.10 ± 0.01	0.10 ± 0.00	0.13 ± 0.02	0.34 ± 0.03	0.85 ± 0.24	0.005 ± 0.001	0.04 ± 0.01
6 months R	0.80 ± 0.10	0.09 ± 0.01	0.12 ± 0.01	0.10 ± 0.01	0.33 ± 0.02	0.92 ± 0.17	0.006 ± 0.000	0.03 ± 0.02
12 months R	0.95 ± 0.12	0.10 ± 0.01	0.15 ± 0.02	0.12 ± 0.03	0.43 ± 0.03	1.06 ± 0.08	0.007 ± 0.001	0.03 ± 0.02
35 months R	1.17 ± 0.07	0.13 ± 0.01	0.18 ± 0.04	0.11 ± 0.01	0.44 ± 0.02	1.00 ± 0.18	N/A	N/A
6 months F	0.70 ± 0.11	0.09 ± 0.01	0.10 ± 0.00	0.13 ± 0.01	0.30 ± 0.05	0.75 ± 0.11	0.004 ± 0.001	0.05 ± 0.02
12 months F	0.76 ± 0.07	0.11 ± 0.02	0.14 ± 0.01	0.15 ± 0.03	0.36 ± 0.00	1.20 ± 0.10	0.006 ± 0.001	0.06 ± 0.03
35 months F	0.96 ± 0.14	0.11 ± 0.01	0.13 ± 0.01	0.15 ± 0.01	0.44 ± 0.01	0.93 ± 0.33	0.005 ± 0.001	0.05 ± 0.04

[Degradation]) and the quality of the STR electropherograms were evaluated.

3. Results

The concentrations measured directly after DNA extraction were comparable to the concentrations after ten freezing/thawing cycles for all sample types (Fig. 1). This applies also when comparing the concentrations measured directly after extraction to the concentrations obtained after storage in the refrigerator or freezer for up to 4 weeks (data not shown) as well as after storage (freezer/refrigerator) up to 35 months (Table 1). A couple of samples had evaporated after storage for 35 months in the refrigerator, so no results were obtained for these samples. None of the samples in the storage and freezing/thawing experiments had a qPCR degradation index above 2 (data not shown). Evaluating the quality of the STR results (4 weeks, 12/35 months or ten freezing/thawing cycles) all electropherograms showed complete allele detection with no indication of degradation (data not shown).

4. Discussion

Generally, quantification and STR analysis was not affected by the different treatment/storage conditions investigated, regardless of sample type. Note that we did not investigate DNA quality per se, but rather the impact on analysis of specific markers with sizes up to 400 bp. The results might have been different if evaluated using methods relying on DNA of high molecular weight e.g. library preparation methods for whole genome sequencing. Freezing/thawing experiments have previously been performed by others with similar conclusion for fragment sizes of 30–40 kb [3]. Pajnica et al. experienced DNA loss in forensic samples after long-term storage and suggest that the buffer used to resuspend the DNA might have an effect on the stability [4]. Since our study shows stable results even though most of the samples have been extracted with a one-tube extraction method leaving potential inhibitors in the extract, the quality of DNA stored in a purer buffer (e.g. extracts from a magnetic bead extraction) is expected to be at least as good. In this study most samples consisted of only cell material, but the extracts containing cigarette filter paper also gave stable results.

5. Conclusions

In this study, DNA quantification and STR analysis results from a variety of different samples were not affected by long-term storage in refrigerator or freezer up to 35 months or freezing/thawing up to ten times.

Conflicts of interest

The authors declare no conflicts of interest.

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