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DEGRADATION OF DNA EXTRACTS STORED UNDER DIFFERENT CONDITIONS: WHAT WE KNOW AND WHAT IS NEW

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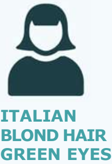
BACKGROUND & AIMS

Preserving the integrity of forensic evidence through the stabilization of the biological signature contained in DNA extracts is particularly important, especially when there is the need of re-analysis samples after a period of time [1]. This translates into the need to appropriately preserve DNA extracts to ensure the successful outcome of forensic genetic analyses, including human identification through STR typing and the application of phenotypic and ancestry panels to infer the morphological characteristics and geographical origin of the contributor [2]. Although the guidelines recommend the freezing of DNA extracts for long-term storage, it is possible that samples are preserved in sub-optimal conditions for a variety of reasons (e.g., faulty freezers, moving samples to the court office) [3-5]. Under these circumstances, little is known about the survival of the DNA and its suitability for future STR and SNP analyses.

MATERIALS AND METHODS

SAMPLE COLLECTION



ITALIAN
BLOND HAIR
GREEN EYES

INDIAN
BLACK HAIR
BLACK EYES

ARTIFICIAL MIXED SAMPLE
1:15 RATIO
[male component : female component]

Buccal swabs from the two subjects were extracted using QIAcube and QIAamp Investigator kits, resulting in a total of 45 female, 45 male, and 45 mixed samples. The extracts were then diluted and aliquoted in 5 replicates each, so that each extract had a DNA concentration of 1 ng/μl and a volume of 20 μl. Storage was assessed under three different conditions: -20°C, +4°C and +20°C.

TIMELINES

The degradation of DNA stored under the three different conditions (5 replicates each) was assessed using the Quantifiler™ Trio DNA Quantification at regular intervals of up to 90 days (7-15-30-90 days). After 90 days, some of the replicates placed at -20°C and +4°C were moved to room temperature and uncontrolled temperature (including replicas at +20°C), breaking the cold chain. Quantification was then repeated 400 days after the start of the experiment and 310 days after the break in the cold chain.
The replicates (male, female and mixture) under all storage conditions with the highest degradation index (or abnormal concentration values) were amplified using the GlobalFiler™ PCR Amplification Kit and sequenced with SeqStudio™ Genetic Analyzer. The resulting STR profiles were analysed using GeneMapper® ID-X v1.5 software.

RESULTS

Given the 90-day results, where storage at +4°C proved to be the least ideal, while storage at +20°C was comparable to storage at -20°C, we decided to proceed with the interruption of the cold chain and resume measurements after an extended period.

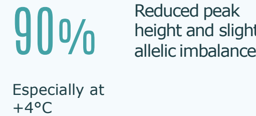
DEGRADATION INDEX (DI)

For all storage conditions analysed, DI<1

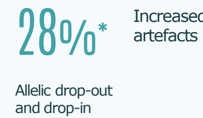
DI<1 when stored at -20°C and +4°C. DI < 2 for storage at +20°C

DI <2 for extracts moved from -20°C to uncontrolled temperature. For all the other condition: DI<1

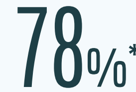
QUANTIFICATION AND ELECTROPHEROGRAM RESULTS



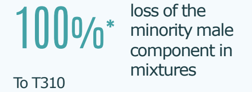
Especially at +4°C



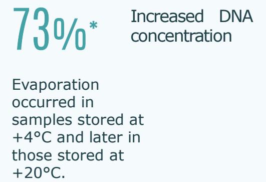
Allelic drop-out and drop-in



Complete profiles at T400 and T310 after relocation



To T310

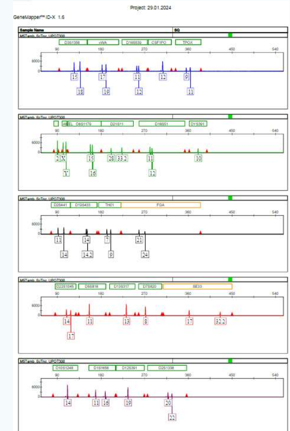


*threshold value of **175 RFU**.

MALE SAMPLE STORED FOR 90 DAYS AT +20°C, FOLLOWED BY 310 DAYS AT UNCONTROLLED TEMPERATURE.

DNA concentration near-complete evaporation	**27,3 ng/μl**
Degradation Index	1,30

Despite the unconventional storage method and the **insufficient volume** available for amplification, a complete, artefact-free male genetic profile was successfully obtained.



CONCLUSION

Surprisingly, DNA extracts did not degrade even after 400 days under any conditions, and the male-female ratio in mixed samples remained unchanged. Storing extracts at +4°C and +20°C led to evaporation, with a concentration increase of up to 20-fold. Evaporation and increased concentration also occurred following the interruption of the cold chain, affecting peak height and equilibrium, with the more significant effects the greater the temperature deviation. These results suggest that the interruption of the cold chain has a greater impact on the DNA preservation that the maintenance of sub-optimal temperatures for prolonged times.

FUTURE PROSPECTIVE

The intention is to proceed with forensic DNA phenotyping and ancestry. SNPs will be analysed using an in-house developed panel, applicable to individual profiles and potentially to mixtures under specific preservation conditions and times.

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