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Preservation of DNA integrity in biological material

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ABSTRACT

We have designed a buffer for preservation of DNA in biological material at room temperature. In this work we evaluated the integrity of DNA from saliva, stored for 4 years and blood stored for 4 months at room temperature. Parallel tests were conducted with other commercial systems. No sign of degradation was detected in the tested samples.

1. Introduction

The collection and preservation of biological material is critical to the field of biomedical research, diagnostics and forensics. Samples often need to be preserved at point of collection so that DNA or RNA analysis can be performed once samples reach the test facilities. The Forensic DNA Laboratory (FDL) at University of the Western Cape) experienced significant degradation while sampling in remote semi-arid locations in South Africa for the intermittent availability of a cold storage facility. To overcome this problem we designed a storage buffer which allows long term saliva storage stored at room temperature [1].

The aim of this project was to evaluate the integrity and quality of human DNA in saliva and blood over long storage periods at room temperature in our buffer. Comparative analysis was conducted using similar commercial products. In addition, the FDL buffer compatibility with common DNA extraction kits was assessed.

Up to date we evaluated saliva stored for 4 years and blood stored for 4 months.

2. Material and methods

A total of 10 saliva samples were collected in the FDL-storage buffer and Oragene OG-500 (DNA Genotek) and stored at room temperature for 4 years. Blood samples were collected from 8 donors in FDL-storage buffer and Sodium Citrate vaccutainers tubes and stored for 4 months at room temperature.

All sample types were extracted using an optimised Phenol Chloroform Isoamyl (PCI) extraction method [2,3] using a sample input volume of 500 µl (250 µl biological sample in 250 µl storage buffer). DNA from saliva stored in Oragene buffer was extracted using the prepIT•L2P kit (DNA Genotek) and saliva stored in the FDL buffer was extracted using Zymo-Quick kit (Zymo Research). All blood samples were extracted using the QIAamp kit (Qiagen).

DNA purity ratio 260/280 nm was estimated using a Nanodrop 2000 (Thermo Fisher) and integrity was evaluated with 0.7% agarose gel electrophoresis. DNA was quantified using the Qubit™ dsDNA BR kit using the Quibit ®Flourometer 2.0 (Thermo Fisher). The Investigator Quantiplex qPCR kit (Qiagen) was used to detect the presence of PCR inhibitory components using a Rotor Gene Q (Qiagen) following the analysis of the internal positive control cycle threshold (*IPCCt*) [4]. All samples were amplified using GlobalFiler Express kit (Thermo Fisher) on a Veriti thermal cycler (Thermo Fisher) following the manufactures conditions [5]. PCR amplicons were separated by capillary electrophoresis on a 3500 Genetic Analyser (Applied Biosystems) and the results were analysed with GeneMapper® data analysis software ID X v1.4 (Thermo Fisher).

Comparison between essays were conducted using standard two sided *t-tests* in microsoft excel.

3. Results and discussion

As shown in Fig. 1A, the average DNA yield obtained for saliva stored in the Oragene device was 3.5 µg ± 0.98. This was significantly higher than the average yield (0.7 µg ± 0.35) obtained for the FDL-buffer extractions (*t-test*, $P < 0.05$). The average DNA yield obtained for blood samples stored in the FDL-buffer was 2.6 µg ± 0.866 for all extraction methods (Fig. 1B). For blood exactions a significant difference in yield was obtained between the FDL-buffer and the Sodium

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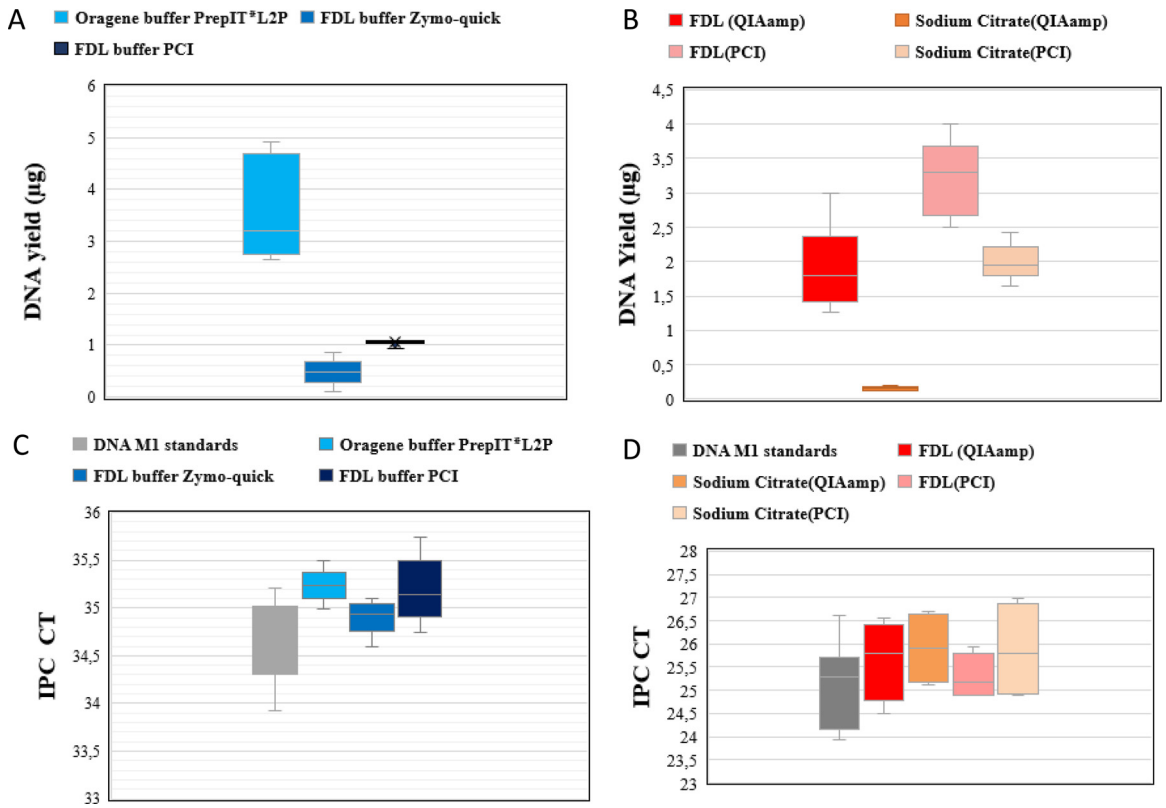

Fig. 1. Box-plots indicating quantitative and qualitative assessments for biological samples stored at room temperature.
A) DNA yield for saliva samples stored for 4 years in the FDL-storage buffer compared to Oragene OG-500, B) DNA yield for Blood samples stored for 4 months in the FDL-storage buffer compared to Sodium Citrate. C) *IPCCt*values for saliva stored for 4 years in the FDL-storage buffer compared to Oragene OG-500, D) *IPC Ct* Blood stored for 4 months in the FDL-storage buffer compared to Sodium Citrate. The DNA M1 standards quantity ranged from 50–0.019 ng/µl with *IPC Ct* values of (25 ± 1.0). The fifty percentile indicates the median and the whiskers indicates standard deviation round the upper and lower quartile

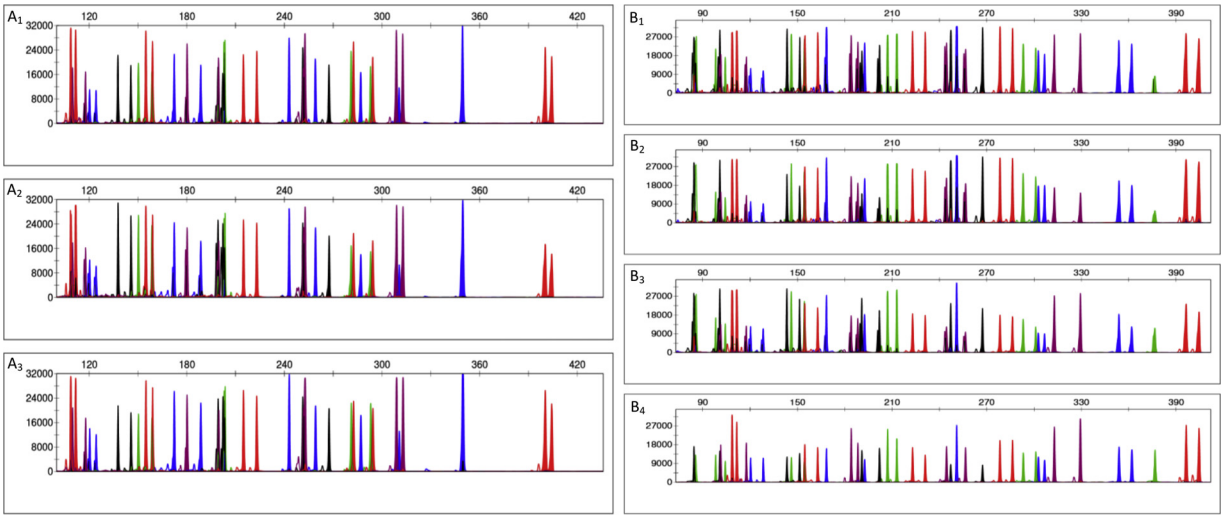

Fig. 2. Global Filer DNA profiles for saliva storage at room temperature after 4 years (A) and for blood storage at room temperature after 4 months (B).
A1) Oragene prepIT•L2P, A2) FDL buffer PCI, A3) FDL buffer Zymo-quick extracted DNA, B1) FDL buffer (QIAamp), B2) FDL buffer (PCI), B3) Sodium Citrate (QIAamp) and B4) Sodium Citrate (PCI).

Citrate storage method (*t-test*, $P < 0{,}05$).

Using the qPCR investigators quantiplex assay, *IPC Ct* values were compared relative to the M1 DNA standards to assay the presence of potential inhibitors and co-eluting impurities. In Fig. 1C, the *IPC Ct* for saliva extracts were within the rage of the controls with the expectation of one outlier which gave a major shift. Similar inferences were made in Fig. 1D for blood, for which no major inhibitory effects were observed. As we previously observed for 2 years room temperature storages [1], the 260/280 purity ratios were within the optimal range for saliva and blood samples (data not shown). DNA profiling confirmed the quality DNA extracts obtained for all sample types. In Fig. 2, full reproducible profiles were obtained without allele drop-out or non-specific artifacts. Amplification of smaller to larger alleles gave homogenous peak heights across the spectrum suggesting no signs of major DNA degradation.

4. Conclusion

We have shown that the FDL-storage buffer has the capability to preserve the integrity of DNA in saliva for 4 years and blood for 4 months, rendering DNA of quality for forensic or database applications. We present a development that can eliminate the need for cold storage during sample collection and can be used for long term room temperature storage for human biological material.

Declaration of competing interest

None

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