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Research Article

Title: Comparative Analysis of DNA Degradation in Whole Blood and Saliva Using PCI Extraction and Basic Molecular Techniques

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Abstract

DNA degradation is a major problem in forensic and clinical genetics, especially when biological samples are stored for long periods under non-ideal conditions. In this study, two routinely used matrices, EDTA whole blood and unstimulated saliva, were compared for their ability to preserve amplifiable DNA during medium-term storage. Samples were collected from healthy volunteers and stored at 4 °C, and genomic DNA was extracted at three time points: fresh (Day 0), after approximately two months, and after approximately three months. A single phenol–chloroform–isoamyl alcohol (PCI) extraction protocol was used for all samples. DNA yield and purity were evaluated spectrophotometrically (A260/A280), and integrity was assessed by 1 % agarose gel electrophoresis. Whole blood provided higher and more stable DNA yields (from about 208 ng/µL at Day 0 to about 117 ng/µL at 3 months) than saliva (from about 94 ng/µL to about 49 ng/µL), with less pronounced decline in A260/A280 ratios. Gel images showed progressive loss of high-molecular-weight bands and increased smearing with storage time in both matrices, but degradation was clearly milder in blood than in saliva. The findings indicate that PCI extraction remains a useful low-cost method for recovering genomic DNA from common forensic sample types and that, under basic refrigerator storage, whole blood is more robust than saliva for preserving amplifiable DNA for 2–3 months.

Keywords:

DNA Degradation; Whole Blood; Saliva; PCI Extraction; Agarose Gel; A260/A280; Forensic Samples

Introduction

DNA is the primary carrier of genetic information and the starting point for nearly all molecular and forensic analyses. At the same time, it is chemically unstable, undergoing continuous spontaneous damage even under moderate conditions. Lindahl (1993) showed that the primary structure of DNA decays by processes such as depurination, depyrimidination and deamination, and Lindahl and Nyberg (1972) quantified depurination in native DNA, demonstrating that purine bases are lost at measurable rates and that abasic sites and strand breaks accumulate over time. Exposure to heat, humidity, oxygen

and UV light accelerates these reactions, leading to extensive fragmentation and base modification (Pääbo et al., 2004). Studies of ancient DNA from bones and teeth have illustrated what long term uncontrolled degradation looks like: typically very short fragments, often under 200 bp, along with characteristic miscoding lesions, especially cytosine deamination near fragment ends (Hofreiter et al., 2001; Pääbo et al., 2004). In forensic and clinical contexts, DNA degradation is also strongly influenced by the nature of the sample and how it is handled. Whole blood contains hemoglobin, nucleases and other components that can catalyse oxidative damage and inhibit PCR if not adequately removed (Al-Soud & Rådström, 2001). Saliva and buccal swabs, while easy and non-invasive to collect, frequently contain lower numbers of host cells and high microbial load, adding more nucleases and potential inhibitors. Pre analytical variables such as fixation, freezing, storage temperature and duration can significantly change DNA quality and the success of downstream tests (Lehmann & Kreipe, 2001; Santos et al., 2018). If these variables are not properly controlled, there is a risk of reduced sensitivity, false negative results or complete failure of advanced methods such as next generation sequencing.

Simple laboratory tools remain important for evaluating DNA degradation in practice. Agarose gel electrophoresis can readily distinguish intact high molecular weight DNA from degraded, smeared material and gives a first visual indication of damage (Opel et al., 2010). Spectrophotometric measurements of A260 and A280 provide estimates of DNA concentration and purity; A260/A280 ratios around 1.8 typically indicate relatively low protein contamination, while lower values suggest co extracted protein or phenol and higher values suggest RNA (Santos et al., 2018). In addition, the PCR amplifiability of short and long targets can be used to “read” the functional integrity of the template, with short amplicons often remaining detectable even when longer ones fail (Opel et al., 2010; Pääbo et al., 2004).

The phenol–chloroform–isoamyl alcohol (PCI) protocol is one of the classical methods for genomic DNA extraction. Although commercial kits have become popular because they are faster and simpler, PCI is still widely used in research laboratories, particularly in settings where cost is a serious limitation (Sambrook & Russell, 2001; Demeke & Jenkins, 2010). When properly executed, PCI can remove proteins and other inhibitors very effectively and generate high molecular weight DNA that works well in PCR and sequencing, even from difficult matrices like blood (Al-Soud & Rådström, 2001).

The present study uses a PCI based extraction protocol and basic molecular tools to compare DNA degradation in two commonly used matrices, whole blood and saliva, during medium-term storage at 4 °C. By applying the same extraction chemistry and analytical methods to both matrices at three time points, it aims to provide practical, comparative data on how sample type and storage duration affect DNA yield, purity and integrity.

Materials and Methods

Whole blood and saliva were collected from healthy adult volunteers after verbal informed consent. For whole blood, 3–5 mL of peripheral blood was

drawn aseptically by venipuncture into EDTA coated vacutainer tubes and gently inverted to mix. For saliva, donors were asked not to eat, drink or smoke for at least 30 minutes before sampling; they then rinsed the mouth with water, waited 5 minutes, and spat approximately 2–3 mL of unstimulated saliva into sterile 15 mL tubes. All samples were labelled and handled with gloves and standard biosafety precautions.

Samples were divided into three storage groups: fresh (Day 0), about two months (60 days) and about three months (90 days). Fresh aliquots were processed within 24 hours of collection. The remaining aliquots of blood and saliva were stored at 4 °C in a laboratory refrigerator, without any additional preservatives. Tubes were kept closed and protected from light, and they were only removed briefly when required.

Genomic DNA was extracted using a PCI-based protocol. A lysis buffer (Solution B) containing 10 mM Tris–HCl pH 8.0, 10 mM EDTA, 50 mM NaCl and 1–2 % SDS was prepared, autoclaved and stored at 4 °C. For each extraction from blood, 200–300 µL of well mixed EDTA blood was placed into a 1.5 mL tube and mixed with 500 µL of Solution B and 10–20 µL Proteinase K (20 mg/mL). Tubes were incubated at 56 °C for 1–2 hours to achieve complete lysis. An equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) was added, mixed by gentle inversion, and centrifuged at around 10,000 rpm for 10 minutes at room temperature. The upper aqueous phase was carefully transferred to a new tube and extracted again with chloroform:isoamyl alcohol (24:1) in the same way.

DNA was precipitated from the purified aqueous phase by adding one tenth volume of 3 M sodium acetate (pH ~5.2) and 2–2.5 volumes of cold absolute ethanol. After gentle inversion, tubes were incubated at –20 °C for at least 1–2 hours and then centrifuged at 12,000 rpm for 10–15 minutes at 4 °C. The supernatant was removed, the pellet was washed with 70 % ethanol, centrifuged again, and the remaining ethanol was carefully discarded. Pellets were air-dried at room temperature until no liquid remained and then resuspended in 30–50 µL TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0). The same procedure was used for saliva, except that 500 µL–1 mL of saliva was first centrifuged at 6,000–8,000 rpm for 10 minutes, and the pellet was used as the starting material.

DNA concentration and purity were measured with a UV spectrophotometer or Nano Drop type device. For each extract, 1–2 µL was loaded on the pedestal, and the absorbance at 260 nm and 280 nm was recorded. DNA concentration was calculated from A260, and the A260/A280 ratio was used as a measure of purity. Where possible, measurements were done in triplicate and mean values calculated. Agarose gel electrophoresis (1 % agarose in 1× TAE) was used to assess DNA integrity. Five microlitres of each DNA sample was mixed with loading dye and run alongside a 1 kb ladder at 80–100 V for about 45–60 minutes. Gels were stained and viewed under UV light, and band intensity and smearing were compared across sample types and storage times.

Results and Discussion

All blood and saliva samples remained visually suitable for analysis throughout the three month storage period. Blood in EDTA tubes darkened slightly after two to three months at 4 °C but did not show visible clots or microbial growth. Saliva tubes showed minor phase separation and mild colour changes, but there was no obvious sign of mould. PCI extraction worked for both matrices at all time points. Fresh and two month samples generally produced compact, visible DNA pellets after ethanol precipitation, whereas pellets from three month samples were smaller and more fragile, which already suggested lower yield.

Spectrophotometric data showed a clear decline in DNA concentration and purity over time. For whole blood, the average concentration at Day 0 was about 208 ng/µL, with an A260/A280 ratio around 1.84, which indicates fairly clean genomic DNA. After approximately two months, the mean concentration fell to roughly 164 ng/µL, and the purity ratio dropped slightly to about 1.79. At three months, the concentration had fallen further to about 117 ng/µL, and the mean A260/A280 was about 1.71, suggesting more protein or other contaminants and/or partial degradation.

Saliva produced lower and more rapidly declining yields. Fresh saliva extracts averaged around 94 ng/µL with an A260/A280 of about 1.81. After two months, this decreased to roughly 72 ng/µL and a ratio of about 1.76. At three months, DNA concentration was around 49 ng/µL and the A260/A280 ratio about 1.67, showing that both yield and purity were compromised. This pattern agrees with the expectation that saliva, which often has fewer host cells and a higher fraction of bacterial DNA and proteins, is more vulnerable to degradation and contamination during storage (Lehmann & Kreipe, 2001; Santos et al., 2018).

Agarose gel electrophoresis confirmed and complemented the quantitative findings. For whole blood, fresh samples showed bright, sharp high molecular weight bands near the wells and minimal smearing, indicating largely intact genomic DNA. After two months, bands were still readily visible but slightly less intense, with a light smear beneath the main band, suggesting early fragmentation. At three months, bands were noticeably weaker and smearing extended further down the lanes, consistent with increased breakage and loss of long molecules.

For saliva, gels revealed more pronounced changes. Day 0 saliva DNA produced visible bands, but they were less intense than for blood, and a small amount of background smear was already present. At two months, band definition worsened and smearing became moderate, and at three months, many lanes showed faint, diffuse bands with heavy smear, indicating that a large proportion of the DNA had been broken down into shorter fragments. These gel patterns are in line with the lower concentrations and reduced A260/A280 ratios recorded by spectrophotometry and support the notion that saliva degrades faster than blood under the same storage conditions.

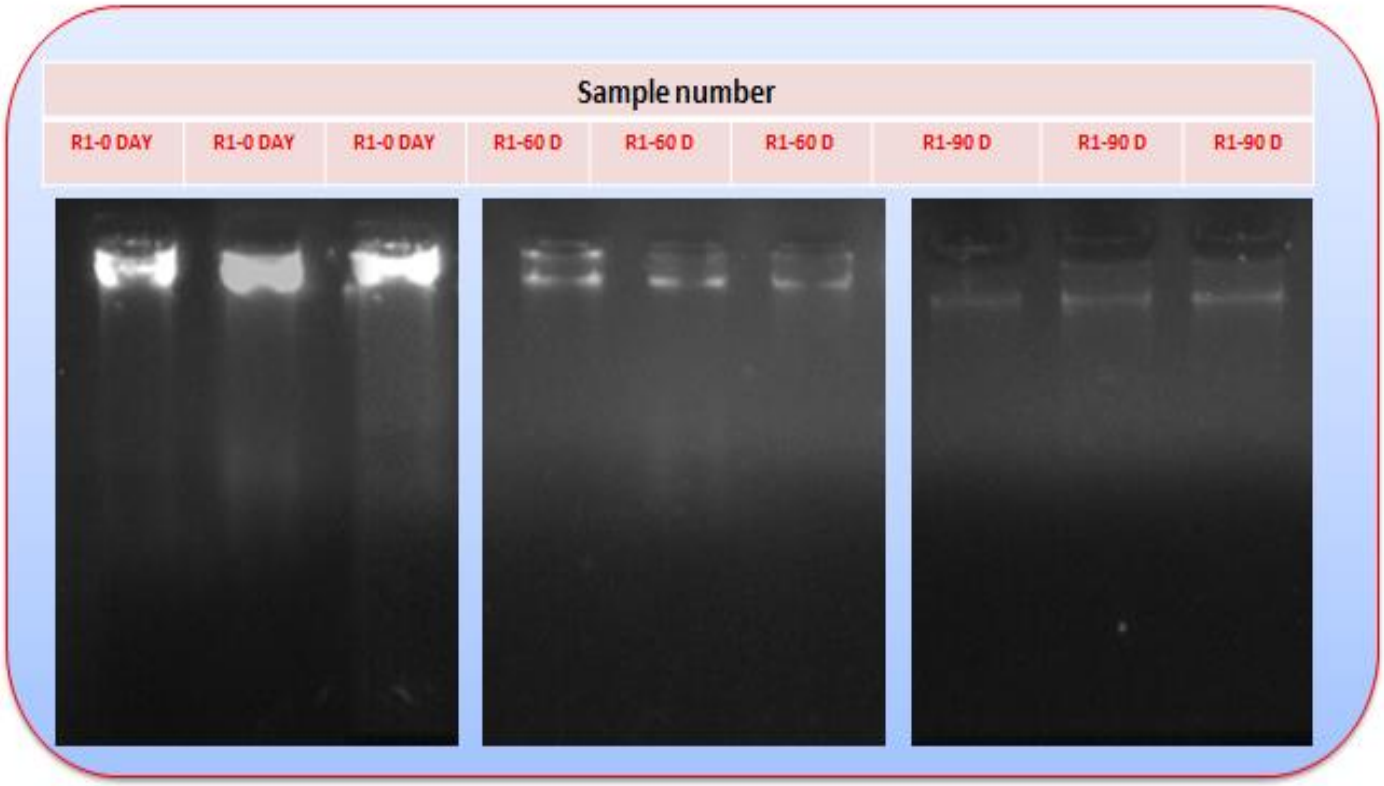

Figure 4.1 . Agarose gel electrophoresis showing DNA whole blood integrity at different storage intervals (Day 0, ~2 months, and ~3 months).

Table 4.1. Genomic DNA from whole blood integrity at different storage intervals (Day 0, ~2 months, and ~3 months).

Storage interval	**Replicate**	**DNA concentration (ng/µL)**	**A260/A280 ratio**
Day 0	R1	210.5	1.85
	R2	205.2	1.83
	R3	208.7	1.84
	Mean ± SD	208.13 ± 2.66	1.84 ± 0.01
~2 months	R1	165.3	1.80
	R2	161.8	1.78
	R3	163.5	1.79
	Mean ± SD	163.53 ± 1.77	1.79 ± 0.01
~3 months	R1	118.6	1.72
	R2	115.4	1.70
	R3	116.8	1.71
Mean ± SD		116.93 ± 1.31	1.71 ± 0.01

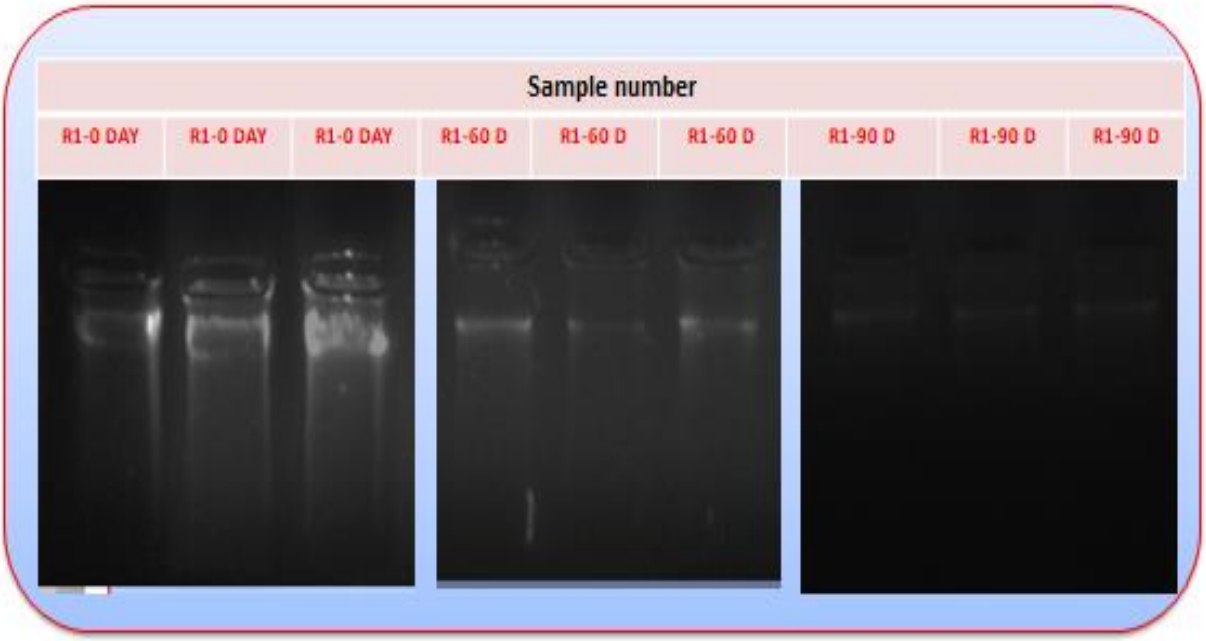

Figure 4.2 . Agarose gel electrophoresis showing DNA saliva integrity at different storage intervals (Day 0, ~2 months, and ~3 months).

Table 4.2. Genomic DNA from salivaintegrity at different storage intervals (Day 0, ~2 months, and ~3 months).

Storage interval	**Replicate**	**DNA concentration (ng/µL)**	**A260/A280 ratio**
Day 0	R1	95.4	1.82
	R2	92.7	1.80
	R3	94.1	1.81
	Mean ± SD	94.07 ± 1.35	1.81 ± 0.01
~2 months	R1	72.3	1.77
	R2	70.8	1.75
	R3	71.5	1.76
	Mean ± SD	71.53 ± 0.76	1.76 ± 0.01
~3 months	R1	49.6	1.68
	R2	47.9	1.66
	R3	48.7	1.67
	Mean ± SD	48.73 ± 0.69	1.67 ± 0.01

Table 4.4. Qualitative agarose gel observations

Sample type	**Storage interval**	**Band intensity**	**Smearing pattern**	**Overall DNA integrity**
Blood	Day 0	Very high	Minimal	Excellent
	~2	High	Mild	Good

	months			
	~3 months	Moderate	Moderate–marked	Partial, degraded
Saliva	Day 0	Moderate	Slight	Acceptable
	~2 months	Moderate–low	Moderate	Noticeable degradation
	~3 months	Low	Extensive	Poor

When both matrices are considered together, a consistent picture emerges. Higher DNA yields and near-ideal A260/A280 ratios in fresh samples match bright, compact bands on gels, while lower yields and poorer ratios in older samples coincide with fading bands and increased smearing. The parallel behaviour across these independent indicators supports that they are reflecting the same underlying process: progressive time-dependent DNA degradation driven by inherent chemical instability and storage conditions (Lindahl, 1993; Pääbo et al., 2004; Bonnet et al., 2010). At the same time, it is important to note that even after three months, neither matrix was completely "empty". Both blood and saliva still contained measurable DNA and visible bands on gels, though more fragmented. In forensic practice, this means that if assays are designed around very short PCR targets (such as mini STRs or small qPCR amplicons), useful genetic information can often still be obtained from such partially degraded templates (Opel et al., 2010; Santos et al., 2018). However, attempts to amplify long fragments or to perform high quality whole genome analysis would be much more likely to fail or to produce biased results.

Conclusion

This study compared DNA degradation in EDTA whole blood and unstimulated saliva stored at 4 °C for up to three months, using a common PCI extraction protocol and basic molecular assessments. Whole blood consistently yielded more DNA and preserved higher integrity over time than saliva. Both spectrophotometric and gel based observations showed a progressive decline in yield, purity and fragment size with increasing storage duration, with the effect being more severe in saliva. These results fit well with known principles of DNA chemistry and matrix dependent behaviour and demonstrate that classical PCI extraction, combined with simple quality checks, can still provide meaningful, practical information in a forensic laboratory. From a casework point of view, the findings suggest that for medium term storage without special preservatives, whole blood is the more reliable matrix when high quality DNA is needed. Saliva remains valuable for non invasive sampling but should ideally be processed more quickly or stored under more protective conditions if long term DNA preservation is required. In both cases, targeting shorter PCR amplicons will increase the chances of success when working with partially degraded DNA.

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Conflict of Interest: None.

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