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A comparison of human blood preservation methods in DNA extraction protocol

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Abstract

Background: Blood preservation method plays an important role in DNA extraction. The current study was conducted at the laboratories of the applied medical sciences college/ University of Karbala during the period from March to May 2021.

Materials: Ten Fresh venous blood samples were collected from healthy males (20-45 years old) by venepuncture and stored at 2–8°C until used. Each particular sample was subjected to five blood collection options as follows: the blood was freshly aliquoted without any treatments, the blood was collected in EDTA (ethylene diamine tetra acetic acid) tubes, the blood was collected with the addition of phosphate buffered saline (PBS) in a ratio of 1:2, the blood was added to PBS 1:2 and Triton X 100, and the blood was centrifuged and then PBS 1:2 and Triton X 100 were added. The quantity and integrity of the extracted DNA were evaluated by agarose gel electrophoresis. The quantity and purity of the DNA were measured using a NanoDrop Spectrophotometer.

Results: The current study indicated statistically significant difference ($p \leqslant 0.05$) among the treated groups when the concentration of the extracted DNA was taken into consideration. There was a statistically significant difference among the five groups when their absorbance ratio was measured at 260 nm/ 230 nm ($p \leqslant 0.05$). However, no statistically significant difference ($p \leqslant 0.05$) was obtained among the groups when the absorbance ratio of 260 nm/280 nm was considered.

Conclusion: The pretreatment of blood samples with buffers (PBS containing 5% Triton X100) prior to DNA extraction can lead to an altered DNA yield and purity.

Introduction

The growing use of genomic sequencing studies in evolutionary theory and disease research necessitates the creation of vast amounts of pure genomic DNA free of protein and RNA contaminants [1]. Polymerase chain reaction (PCR), restriction endonucleases analysis, genotyping, variant detection, genetic anomalies identification, epigenetic analyses, and a variety of preventive and diagnostic studies, all require the use of DNA of high quality [2]. Furthermore, when the DNA extraction improves in terms of speed and cost, it will become much more study friendly. Blood among different sources used for DNA extraction has become increasingly important in biological studies. Blood has become a crucial component of hematology, biochemistry, clinical research, and forensic studies. Because of the existence of white blood cells (WBCs) in blood, it has become a major source for genomic DNA [3]. The relevant components of biological samples, including blood, may be extracted after storage, and the best yield of those components is reached, due to efficient and effective processing of the samples. The faster the samples are processed, the higher the quality of the interested components extracted. Examples of the basic blood processing methods include freezing and splitting or separating blood into serum and clot [4]. The current study aimed to investigate the best processing conditions applied for blood samples before DNA extraction that can ultimately lead to higher DNA yield and purity.

Methods

The current study was conducted at the laboratories of the college of Applied Medical Sciences /Karbala University, during the period from March to May 2021. All the procedures were per-formed according to the standard ethical guidelines. Ten Fresh venous blood samples were collected from healthy males (20-45 years old) by venipuncture. Each particular blood sample (200 µl) was subjected to five blood collection options as follows: the blood was freshly aliquoted (group T1), the blood was collected in EDTA (ethylene diamine tetra acetic acid) tubes (group T2), the blood was collected with the addition of PBS in a ratio of 1:2 (group T3), the blood was added to PBS in a ratio of 1:2 containing 5% Triton X 100 (group T4), the blood was centrifuged at 1,000 xg for 5 minutes at 4 °C, the supernatant was removed, and the blood was added to PBS in a ratio of 1:2 containing 5% Triton X-100. The gSYNC™ DNA Extraction Kit (Geneaid®) was utilized to obtain DNA from the blood samples of the participants. The procedure strictly followed the manufacturer's instructions. The quantity of isolated DNA was evaluated by agarose gel electrophoresis. Three microliters of each DNA sample were electrophoresed on a 1.5% agarose gel stained with 5µl RedSafe, and the bands were visualized by UV transillumination. The concentration and purity of the extracted DNA were measured using a Spectrophotometer (NanoDrop) (Thermo Scientific, Inc.). Protein contamination was measured by the estimation of the 260/280 nm absorbance ratio, whereas 260/230 nm ratio was used to determine contamination by sugars, salts, and organic reagents.

Statistical Analysis

Statistical analysis of data was done by SPSS 24. Mean nucleic acids concentrations were obtained from the Nanodrop measurements as well as the absorption ratios of A260 nm/A230 nm and A260 nm/A280 nm. Significant differences were analyzed by Tukey's honestly significant difference and ANOVA (Post Hoc test).

Results

The current study indicated statistically significant differences ($p \leqslant 0.05$) among the groups of the study when the concentration of the extracted DNA was taken into consideration after applying different treatments for the collected blood. A statistically significant difference was observed when the mean of nucleic acid concentration of T1 (34.264±3.05) was compared with that of T2 (42.851±3.21); the same findings were also obtained when the comparisons involved T1 with T3 (44.65±1.00) and T1 with T5 (45.03±0.88). However, comparisons between the treated groups showed no statistically significant differences (Figure 1).

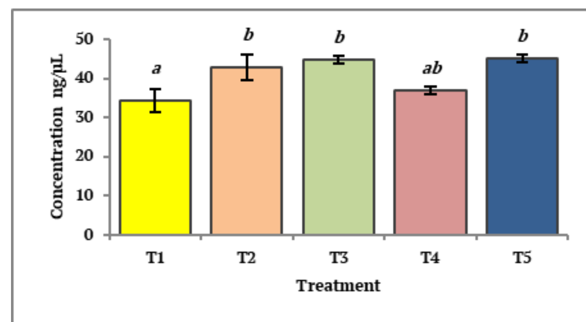

Figure 1: The mean concentration of the extracted nucleic acid after various treatment options. The different letters (a–b) above the bars indicate significant mean differences among each treatment group, according to Tukey HSD Post Hoc ($p \leq 0.05$). The values are the means of ten replicates. Mean values are indicated by vertical bars ± SE of means.

Furthermore, the current work demonstrated presence of a statistically significant difference when comparing the five study groups with each other based on the ratio of absorbance calculated at 260 nm/ 230 nm ($p \leqslant 0.05$). The findings also indicated a significant difference when

pairwise comparisons within ANOVA data were exploited. The comparisons of T1 (1.192±0.15) with T3 (1.935±0.04), T1 with T5 (1.99±0.047), and T2 (1.49±0.05) with T5 (1.99±0.047) revealed statistically significant difference ($p \leq 0.05$) from the one hand, but all other comparisons showed no statistically significant difference from the other hand (Figure 2).

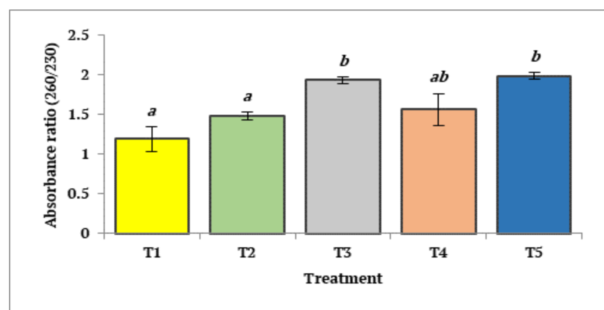

Figure 2: The absorbance ratio measured at 260 nm and 230 nm. The different letters (a–b) above the bars indicate significant mean differences among each treatment group, according to Tukey HSD Post Hoc ($p \leq 0.05$). Mean values are indicated by vertical bars ± SE of means.

This study also revealed that the highest absorbance ratio (1.838±0.018) measured at 260 nm/280 nm was reported in the treated group 2 (T2), while the lowest absorbance ratio (1.78±0.018) was in the treated group 5 (T5). No statistically significant differences were obtained when all study groups were compared to each other ($p \leq 0.05$) (Figure 3).

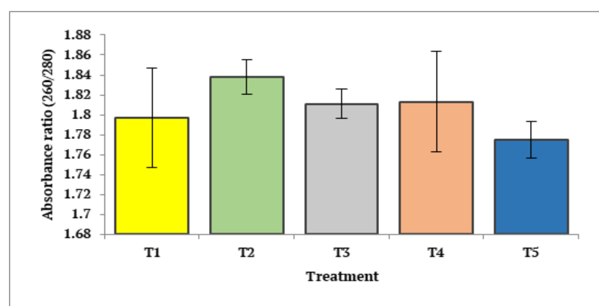

Figure 3: The absorbance ratio measured at 260 nm and 280 nm. the different letters (a–b) above the bars indicate significant mean differences among each treatment according to Tukey HSD Post Hoc ($p \leq 0.05$). Mean values are indicated by vertical bars ± SE of means.

Discussion

The integrity of DNA samples is critical for genetic studies to detect human diseases and examine gene activity *in vivo* [5]. For studying genetic diseases, DNA extraction, particularly from human leukocytes, and recovery after long-term storage are crucial [6]. DNA polymorphism research necessitates the use of intact and high-quality DNA. DNA integrity is influenced by a number of parameters, including storage temperature and hydration buffer. Both the quantity and quality of the DNA obtained can be affected by the physical and chemical treatments used in DNA extraction [7]. The compatibility of the storage buffers with all subsequent analyses, including the extraction procedure, is critical for choosing the best storage buffers [8]. Ethylene diamine tetra acetic acid (EDTA) is a chelating agent can bind to metals via four carboxylate and two amine groups. It is frequently employed in detergents [9], food industries [10], and as an anticoagulant, among other applications in molecular biology, due to its great complexing ability for most metal ions [11]. When DNA was dissolved in large quantities of EDTA, it remained intact and did not degrade for long periods of time [12]. EDTA vacationers have been found to be the best for storing whole blood samples for long periods of time in a frozen state in a few trials [13]. Phosphate buffered saline solution contains a sodium chloride, sodium phosphate, and potassium phosphate salt that prevents osmosis by balancing the salt content around cells. In biological laboratories, PBS and similar buffered solutions are commonly used for cell washing, immunohistochemistry, and other cellular techniques [14]. Triton X-100 (TX100) is a non-ionic solvent that is often used to lyse cells to obtain cellular components, especially the proteins, as well as to permeabilize live cell walls for transfection [15]. However, if high volumes of Triton X100 are added or the cells are subjected to Triton X100 for an extended period of time, the cells will die [16].

To acquire a highest amount of genomic DNA from blood samples of goat, some researchers reported utilizing various detergents from different manufacturers (Arial, Tide, Active wheel, Rin, Henko stain Champion, and Ezee) [17]. The results of the present work were in accordance with a previous report in which a significant alteration in the quality of the extracted DNA was achieved when Triton X buffer was used [12]. Furthermore, prior findings showed that the DNA extracted using the detergent approach yielded much more DNA than the standard Phenol: Chloroform: Isoamyl alcohol method [18].

Various sample pre-treatment options, including PBS, EDTA, and Triton X reagents can affect the quality of the extracted DNA, thus leading to a more fruitful utilization of the sample for high throughput applications and extensive downstream processing. It appeared that the pretreatment of blood samples with PBS containing 5% Triton X100 prior to DNA extraction can improve the DNA yield and purity.

Competing Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Authors' Contribution

All authors contributed equally to this study.

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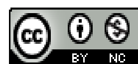

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