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DNA Isolation: A Method for Improving the Efficiency of DNA Extraction from Clotted Blood Samples

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A B S T R A C T

In general, the genome research of high DNA extraction from clotted blood produced a low quality. The aim of this study is to develop a simple and safe technique for dispersing blood clots with ball-bearing metal shots. A total of 31 clotted blood samples were randomly selected as part of the Mashhad Stroke and Heart Atherosclerotic Disorders (MASHAD) study. Ten milliliters of peripheral blood were collected in plain tubes. Samples were spun, and the serum was separated. All tubes containing clotted blood were stored at −80°C. Another 32 samples of clotted blood from this cohort were chosen and considered as a comparator group. The yield and purity of DNA obtained by the three steps were significantly different ($P < 0.0001$), with a higher yield in the modified salting-out method. In conclusion, the salting-out method is simple, efficient, and economical for DNA isolation from old clotted blood samples.

1. Introduction

The increasing demand for genome-based analyses in modern evolutionary and disease research have also increased the need for a bulk amount of pure DNA genomics.[1,2] DNA can be extracted from many biological samples such as hair, blood, semen, saliva, skin cells, and many more. The one that has gained astounding importance in biological research is blood. Blood has become an integral part of biochemistry, hematology and clinical studies, and forensic investigations. DNA extracted from blood samples is crucial for the confirmation of genetic abnormalities, as well as application in epigenetic studies and preventive medicine. It serves as an important source of genomic DNA because of the presence of nucleated white blood cells. There are many methods of DNA extraction, which are phenol-chlorom extraction, salting-out, silica guanidinium thiocyanate, and commercial kits are commonly used5, but it usually comes with high cost.[3,4]

Salting-out method is a simple, non-toxic, and inexpensive method for extracting high-molecular-weight DNA from peripheral lymphocytes. The optimal concentrations of various salts such as Tris- HCl, KCl, MgCl2, and NaCl are used as the buffer to obtain favorable DNA results.[5] This study aimed to explore the efficient technique for the fragmentation of the clot before DNA extraction processing.

2. Methods

Sample collection

A total of 31 clotted blood samples were randomly selected as part of the Mashhad Stroke and Heart Atherosclerotic Disorders (MASHAD) study. Ten

milliliters of peripheral blood were collected in plain tubes. Samples were spun, and the serum was separated. All tubes containing clotted blood were stored at −80°C. Another 32 samples of clotted blood from this cohort were chosen and considered as a comparator group.

Sample preparation

Before proceeding to DNA extraction using a modified salting-out method and modified QIAamp® DNA Blood Midi Kit, 500 µL phosphate-buffered saline (PBS) (1X) and two ball-bearing metal shots, autoclaved in 121°C for 15 minutes were added to each tube of clotted blood sample and were gently rotated in an electric laboratory rotator for 1 hour at room temperature (18-25°C). In QIAamp® DNA Blood Midi Kit, blood samples were thawed for 1 hour at room temperature (18-25°C).

Modified salting-out method

Two milliliters of blood clot were transferred to a tube containing 7 mL of cell lysis buffer solution (CLB) (0320 mmol/L sucrose, 10 mmol/L Tris-HCl, 2 mmol/L MgCl2, 1% Triton X-100, 4 mmol/L trisodium citrate dehydrate) (pH 6.50) (Merck, Germany). The tubes were mixed well by pulse vortexing for 2 minutes. After centrifuging at 3800 rpm (1533g) for 10 minutes, the supernatant liquid was carefully discarded to waste, and 5 mL CLB has added to the pellet again. Tubes were well shaken for 2 minutes and centrifuged at 3800 rpm (1533g) for 10 minutes. After the supernatant was discarded, 5 mL of low salt buffer containing 10 mmol/L Tris-HCl, 4 mmol/L MgCl2, and 10 mmol/L KCL (known as TKM1) and 0.1 mmol/L Na2EDTA, pH 4.46 (Merck, Germany), was added to the pellet.[6,7]

The samples were mixed for 1 minute and centrifuged at 3800 rpm (1533g) for 10 minutes at room temperature. After decanting the supernatant, 1.5 mL of high salt buffer TKM2 containing 10 mmol/L Tris-HCl, 4 mmol/L MgCl2, 8 mmol/L KCL, and 1 mmol/L Na2EDTA, 390 mmol/L NaCl, pH 4.82 (Merck, Germany), along with 100 µL solution of 10% sodium dodecyl sulfate (SDS) (w/v) was added to each tube. The samples were then mixed for 1 minute. This mixture was incubated for 1 hour at 65°C. Then, 500 µL of 6 M NaCl (Merck, Germany) was added. After vigorous shaking for 15 seconds, proteins were removed by centrifugation at 3800 rpm (1533g) for 10 minutes. The supernatant liquid was carefully transferred to a new clean tube containing 4 mL cold absolute ethanol (Merck, Germany). The tubes were inverted several times gently, causing long strands of high-molecular-weight DNA to appear. The DNA was transferred to a 1.5-ml sterile microtube along with the addition of absolute cold ethanol. If the DNA cloud was not seen, the solution was transferred to a 1.5 mL sterile microtube centrifuged at 14000 rpm (20817g) for 2 minutes, and the supernatant was then discarded. These steps were repeated several times until no supernatant solution remained in the tube. The pellet was washed once with 300 µL of 70% ethanol (Merck, Germany) and then centrifuged at 14000 (20817g) rpm for 2 minutes. The DNA pellet was allowed to be dried for at least 10 minutes at room temperature (18-25°C) until there was no trace of ethanol. Finally, the DNA was dissolved in 100-200 µL of sterile distilled water before storage at -20°C.[8,9]

Modified QIAamp® DNA blood midi kit

A 500 µL phosphate-buffered saline (PBS) (1X) and two ball-bearing metal shots were added to each tube of the clotted blood sample. They were then gently rotated for 1 hour. DNA extraction of the clotted blood samples was performed with the QIAamp® DNA Blood Midi Kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations.[10]

QIAamp® DNA blood midi kit to compare the results A 22 frozen clotted blood samples as the control group was chosen from the MASHAD cohort and thawed at room temperature (18-25°C) for 1 hour, followed by DNA extraction, which was performed using QIAamp® DNA Blood Midi Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

Statistical analysis

This study used SPSS 17.0 (SPSS Inc, Chicago, Illinois) for statistical analysis and used the Shapiro-Wilk test for the data normality. Non-normally distributed data are presented as median and percentiles (IQ1- IQ3). The Kruskal-Wallis test was used for comparing non-normal data among the groups. Statistical significance was set at $p < 0.05$.

3. Results and Discussion

The mean result and purity of DNA obtained by these three methods were significantly different ($P < 0.001$), and a significant difference was also observed in the A260/A280 ratios among those methods ($P = 0.01$). The quality of the DNA was fairly uniform in both modified salting-out and modified QIAamp ® DNA Blood Midi Kit methods. DNA purified from clotted blood samples could be used to amplify and genotype the exon 3 of the crystalline gamma-D (CRYGD) gene, and SNP rs1333049 successfully showed by the analysis of PCR products and real-time PCR.

An alternative method was developed in this study for the isolation of high-quality DNA with a large number of blood clots, which use of the ball bearing metal shots to homogenize and break down the blood clot and add phosphate-buffered saline (PBS) to maximize the volume of blood in liquid form that belief will increase the buffy coat volume.[11] The higher quantity of DNA using a mechanical device showed by the increase of DNA extraction in the modified salting-out method and modified QIAamp® DNA Blood Midi Kit looks significant when compared with QIAamp® DNA Blood Midi Kit ($p<0.001$, 75.43 ± 47.12 and 91.48 ± 63.07 vs 42.46 ± 26.21). This observation is consistent with previous results, which indicated a mechanical breakage of the clots is useful for enhancing the amount of DNA.[9,12]

The use of ball-bearing metal shots in blood clot-containing tubes is a simple and safe mechanical device to break down the blood clot. With a minimized handling of the clotted sample, the risk of possible external contamination of the samples can be reduced, improving the DNA yield through the efficient disintegration of the clot, speeding up the extraction procedure as blood clots may hinder the conventional extraction procedures, and improving the safety are the superiority in the use of ball bearing metal shots. PCR genotyping was successfully extracting the DNA. Real-time PCR is more accurate in gDNA quantification. An inhibitory factor is not identified in the solution. These findings demonstrated the quality and quantity of DNA samples purified from clotted blood. This method would also help to use frozen blood clots as a source of DNA in many areas of molecular biology.[7-9]

Pipetting each step to dissolve the pellet is challenging. Modified QIAamp® DNA Blood Midi Kit is a suitable choice for enhancement of the speed of DNA purification and minimizes the possibility of cross-contamination. The reduction time for DNA extraction and quality improvement and quantity of DNA is fairly reduced, as shown by using physical and chemical methods.

4. Conclusion

A modified salting-out method for DNA isolation from frozen blood clots is simple, safe, and low-cost in routine laboratory tasks. The utilization of the ball-bearing metal shots for the fresh blood as a homogeneous suspension may be skipped, although this method is beneficial and practical for either fresh or clotted blood.

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