

# Assessing the Quality and Quantity of DNA Isolated from Coagulated Blood Samples Using Five Extraction Methods

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## Abstract

Blood DNA extraction is one of the vital steps in various DNA-based analyses. However, one of the challenges is processing clotted blood samples. Oftentimes these protocols are time consuming and use hazardous organic solvents. Many researchers have also resorted to commercial kits to circumvent the need to optimise different methods. However, manual methods may need to be employed due to cost consideration. Therefore, this study was conducted to assess various extraction methods to determine the best method that can provide DNA that has high quality, integrity, and purity, and the most time and cost-efficient method. Samples of coagulated and un-coagulated blood were collected and extracted using four published manual methods and a commercial kit. Analysis of the extracted DNA's quality and quantity was done using gel electrophoresis and biophotometer. Among the methods compared, DNA extracted using a method proposed by Moradi et al. (2014) [1] was the best as it yields the best DNA in terms of quantity and purity, and the most time and cost effective.

**Keywords:** DNA, Extraction, Genomic DNA, Clotted Blood.

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## INTRODUCTION

The use of DNA-based analysis is instrumental to advances in genomics and molecular biology, and one of the key sources of DNA is the extracted genomic DNA from blood. However, one of the challenges is the clotting of blood due to improper handling or prolonged storage.

A study that collects blood often uses uncoagulated whole blood collected in tubes for DNA extraction. Once the DNA is extracted from that sample, the remaining or compacted blood will be discarded, which is a waste of resources. Although clotted blood is a source of DNA, the extraction process is quite cumbersome [2]. Clotted blood renders the sample difficult to work with because it resembles tissue rather than liquid blood. This condition may affect the condition of the extracted DNA in terms of its quality and quantity.

Various mechanical and chemical methods are available to extract DNA from clotted blood, with varying quality and quantity [3]. However, some of these methods could result in DNA with poor quality and low quantity, which could lead to incomplete data set and impacting the outcome of the study [4]. Furthermore, technologies for DNA sequencing require high quantity and quality of DNA [5].

Advantages of extracting DNA from clotted sample include fewer blood draws, minimal use of materials, reducing patient's discomfort and anxiety, and the use of a single

blood draw can reduce the number of venepunctures [6]. This is particularly important in paediatrics cases, whereby multiple venepunctures are often impossible, thus mandating the best practice to be used to provide quality samples for optimal studies [7]. Therefore, this current study aimed to identify a cost-effective method to perform DNA extraction using clotted blood by comparing four published methods and one commercial kit. These methods were assessed in terms of quantity, integrity, and purity, as well as efficiency in terms of cost and time.

## MATERIALS AND METHODS

### Blood sample collection

This study was approved by the CUCMS Research Ethical Review Committee (CRERC). A sample of 10 mL of blood was drawn from a volunteer after obtaining informed consent. Then, the sample was placed in an EDTA tube (uncoagulated) and a plain tube (coagulated) and stored at -80 °C until further use. Method 1 [8], Method 2 [9], Method 3 [1], Method 4 [10], and Method 5 (Kit protocol) were used.

### DNA extraction procedures

#### Method 1 [8] salting out method

0.50 mL of clotted blood was aspirated using a 2 mL disposable serum pipette and mixed with 0.25 mL of Lysis

solution (360 µg/mL proteinase K, 150 mM sodium chloride, 50 mM EDTA, and 2% sodium dodecyl sulphate). The mixture was then incubated at 55-65 °C for 3 hours with periodic mixing. After incubation, it was added with saturated 0.15 mL of NaCl and 0.60 mL of chloroform and vigorously mixed for 10 min. The mixture was centrifuged at 5,000 rpm (5 min). The upper 0.40 mL of the aqueous phase, which contained the DNA, was transferred into a new tube containing 0.80 mL of 90% ethanol. The DNA pellet was obtained via centrifugation at 5,000 rpm (5 min). The DNA pellet was added with 0.10 mL of TE buffer (10 mM Tris-HCl and 1 mM EDTA), after being washed with 70% ethanol. The DNA was stored at 4 °C.

### Method 2 [9]

0.50 mL of blood sample was transferred into a 2 mL microfuge tube. Then, 1 mL of RBC lysis buffer (10 mM Tris-HCl, 320 mM sucrose, 5 mM MgCl<sub>2</sub>, 1% triton X-100, pH 8) was added to the tube. The mixture was placed on ice for 15 min. Then, the mixture was centrifuged at 13,000 rpm (10 min). The upper layer decanted, and 0.5 mL of RBC buffer and 0.5 mL of KCl buffer were added. This mixture was mixed well and centrifuged at 13,000 rpm (5 min). Then, the supernatant was removed. The washing step with RBC buffer and KCl was repeated until the pellet becomes white or pink-white. 0.70 mL of lysis buffer (400 mM Tris-HCl, 150 mM NaCl, 60 mM EDTA, 10% SDS, pH 8) and 0.14 mL of sodium perchlorate was then added to the tube and mixed. The reaction tube was incubated at 65 °C for 90 min. The tube was left for 1-2 min at room temperature before added with 0.10 mL of sodium acetate and 0.50 mL of butanol. The mixture was mixed well by slow inversion and then centrifuged (13,000 rpm, 10 min). The bottom layer was transferred to a new tube and added with 0.12 mL of NaCl, with 2 volumes of iced-cold ethanol. Then the tube was mixed by inversion. Then, the tube was stored at -20 °C for 30-45 min before being centrifuged at 13,000 rpm (15 min). The resulting supernatant was removed, and 0.50 mL of ethanol was added. The tube was inverted gently and centrifuged again for 5 min. The supernatant was discarded and the pellet air-dried until all ethanol has evaporated. The DNA was reconstituted with 0.05 mL of double distilled water and then stored at 4°C.

### Method 3 [1] boiling and salting out

0.50 mL of clotted blood was put into a 2 mL microtube and added with 1 mL of distilled water. It was mixed for 30 sec by inversion and then centrifuged (7,000 rpm, 5 min). The supernatant was removed. Washing with distilled water was repeated for two times. 1 mL of buffer A (red blood cell lysis buffer: 10 mM Tris-HCl, 320 mM sucrose, 5 mM MgCl<sub>2</sub>, 1% triton X-100, pH 8) was added to the pellet and mixed using a vortex mixer. Then, the mixture was centrifuged (7,000 rpm, 5 min) before removing the supernatant. 1 mL of Buffer B (white blood cell lysis buffer: 4 mM Na<sub>2</sub>EDTA, 20 mM Tris-HCl, 100 mM NaCl, pH 7.4)

and 0.10 mL of 10% SDS were added, and the pellet was suspended by using the vortex mixer, or by shaking, and then, the microtube was incubated at 65 °C for 15 min. Next, the microtube was chilled, using ice for 3 min and 0.40 mL of saturated NaCl was added, and the microtube was shaken gently for 30 sec and centrifuged at 5,000 rpm for 10 min at 4 °C. The upper phase of the 1 mL mixture was transferred into a new 2 mL tube and an equal volume of cold absolute ethanol was added. The tube was shaken gently until a white skein of DNA appeared. The tube was centrifuged (1,300 rpm, 10 min, 4 °C) before removing the supernatant. Then the resulting pellet was washed with 1 mL of 70% ethanol (cold). The supernatant was removed after centrifugation (10,000 rpm, 10 min) and the pellet was air-dried at room temperature. The extracted DNA was added with 0.05 mL of double distilled water and then stored at 4°C.

### Method 4 [10] boiling

TE buffer (10 mM Tris-HCl and 1 mM EDTA) (1 mL) was added to 0.50 mL of blood sample. The tube was inverted several times and then, centrifuged at high speed for 5 min. The resulting supernatant was removed. 0.1 mL of 10 mM TE/1% Tween 20 that contained 200 µg of proteinase K was added to the mixture. The mixture was incubated at 55 °C overnight. Then, the mixture was heated at 97 °C for 10 min and centrifuged at high speed for 5 min. The supernatant was removed and placed into a new tube and the DNA was stored at 4°C.

### Commercial Kit

The kit used in this study was the Invisorb® Spin Blood Mini Kit. The sample of blood was processed according to manufacturer's instructions.

### Quality and quantity assessment of extracted DNA

#### Qualitative assessment: Agarose gel electrophoresis

To evaluate the extent of DNA degradation and to analyse the integrity of the DNA, gel electrophoresis was conducted (0.5% agarose gel) using 1 Kb Plus DNA ladder (Invitrogen).

#### Qualitative assessment: Polymerase Chain reaction

The feasibility of the DNA extracted to be used in downstream processing, was evaluated using an in-house PCR method. DNA extracted using the Invisorb® Spin Blood Mini Kit (Stratec Molecular, Germany) was used as control.

#### Quantitative assessment

The concentration and purity of genomic DNA was analysed by determining the 260/230 and 260/280 nm absorbance ratios (A<sub>260/230</sub> and A<sub>260/280</sub>) using NanoVue Plus spectrophotometer (GE Healthcare, UK).

## RESULTS

Table 1 shows the spectrophotometry results for uncoagulated blood and clotted blood samples, using five different extraction methods. According to the results, the highest DNA yield was obtained using Method 3 [1], with DNA concentration of 218.5  $\mu\text{g/mL}$ , followed by Method 4 [10], which yielded 213.0  $\mu\text{g/mL}$  of DNA. Extraction using the commercial kit had resulted in a very low recovery value (DNA concentration = 36.0  $\mu\text{g/mL}$ ). Among the five extracted samples, three methods yielded pure DNA, except for DNA extracted using method 1 [8] (A260/A280: 0.931) and method 4 [10] (A260/A280: 1.030).

Gel electrophoresis results are summarized in Table 2. For samples extracted using Method 1 [8], showed that while there was a good yield from normal blood, the DNA from coagulated blood was degraded. As for the results from using Method 2 [9], the DNA fragments of both samples were clearly visible and indicated a high integrity gDNA. There was minimal degradation observed and high intensity of DNA bands for both B1 and B2, which indicated that this method was able to yield high concentrations of DNA. Electrophoresis of the extracted DNA using Method 3 [1] resulted in high concentration with minimal degradation. Furthermore, the extracted DNA from clotted blood sample showed high molecular weight and integrity, which was comparable with the DNA extracted from uncoagulated blood. Extraction using Method 4 [10] showed that both blood samples were of low concentration and had undergone DNA degradation. The kit protocol yielded DNA with high integrity as there was no degradation, with comparable band intensities for both coagulated and uncoagulated blood samples.

The suitability of the extracted DNA to be used in downstream processing was evaluated using PCR. A comparison was made for the results between samples extracted using Method 3 [1] and commercial kit, as shown in Table 3. The results showed that the DNA extracted using the manual and kit methods were suitable for downstream processing, such as for PCR.

Table 4 shows the comparison of five different extraction methods in terms of purity, yield, time, simplicity, and cost. The best results were observed for Method 3 [1], which yielded a high amount of DNA (218.5  $\mu\text{g/mL}$ ), with 1.868 purity ratio and cheaper than the extraction kit. Although the extraction time required by the commercial kit was shorter (25 min), it was costly and yielded the lowest quantity of DNA (36.0  $\mu\text{g/mL}$ ). On the other hand, Method 4 [10] required the longest time to perform because it involved an overnight incubation.

## DISCUSSION

DNA extraction demands an effective, flexible, and economical procedure that can be applied to clotted blood samples. Moreover, the extraction should yield a high amount of pure DNA. The A260/A280 absorbance ratio was

used to assess protein contamination. For blood samples, the A260/280 ratio that ranges between 1.8 and 2.0 indicates acceptable DNA purity [11]. If the obtained ratio was lower than this range, then it may indicate the presence of phenol, protein, or other contaminants that were absorbed strongly at 280 nm, whereas a higher ratio would indicate the presence of RNA. Additionally, the A260/A230 ratio can also be used as an alternative measure of DNA purity. The expected A260/A230 values are between 2.0 – 2.2. If the ratio was lower than expected, it may indicate the presence of contaminants that were absorbed at 230 nm [12].

Method 1 [8] included a long incubation period (3 hours), with the total process time of approximately 4 hours. One of the advantages of this method was the yielded DNA was higher than the yield using the kit protocol. Method 2 [9] gave the highest concentration of extracted DNA from normal blood. Therefore, this method may be suitable for manual DNA extraction from normal blood if time is not an issue (it requires 2 hours for the whole process). However, this method was used with coagulated blood, it yielded 56% less DNA compared to the yield from normal blood. The use of Method 3 [1] yielded the highest amount of pure DNA from coagulated blood. This method was simple to conduct and was the fastest method (1 hour) compared to the other three manual methods. In addition, this method is also reliable to be conducted in small laboratory setting [1]. Method 4 [10], which employed boiling to disrupt the cells, was complex as it required an overnight incubation and a total processing time of two days. The purity of the DNA extracted using this method was low and the absorbance ratios suggested the presence of contaminants. Lastly, these manual extraction methods were compared with the kit protocol. The kit delivered its guarantee to yield about 10  $\mu\text{g}$  of DNA and the absorbance ratio was recorded at 1.7-2.0. However, the yield was extremely low compared to the yields of the manual methods. According to its manual [13] this kit is preferable for use with whole blood samples as opposed to coagulated samples because they could clog the spin filter membrane, which can result in a low yield of DNA. Although it was extremely simple and time saving, this kit was costly compared to the manual methods.

Gel electrophoresis was used to determine the integrity of the extracted DNA. It separates the DNA molecules according to band size and the integrity of DNA could be assessed qualitatively. Based on the results, DNA with the highest integrity from coagulated blood samples were obtained using Method 2 [9] and Method 3 [1], which indicated high molecular weight DNA. Other methods showed signs of DNA degradation, with the highest relative degree of degradation was when using Method 4 [10]. According to the results in this current study, the integrity of extracted DNA from both coagulated and normal samples were high when using the commercial kit.

The quality of the extracted DNA was also evaluated using the in-house PCR method to determine its suitability for downstream processing and the PCR result was positive.

## CONCLUSION

Specific issues, such as quality, quantity, and efficiency, in terms of cost and time must be considered when processing large volumes of samples. The methods 1 [8] and 4 [10] were not preferable because they were time consuming and yielded DNA of lower quality and quantity. Ultimately, this study had evaluated four manual methods and a commercial kit to identify the most efficient, fast, and inexpensive protocol for high quantity of gDNA extraction from clotted blood as well as uncoagulated blood samples. Among the five methods of extraction that have been evaluated, Method 3 [1] showed positive results for all predetermined criteria. This protocol was fast, efficient, and inexpensive, as well as yielded high volumes of DNA compared to the kit protocol. The resulting DNA was of high integrity and purity, and can be used for further downstream processing, such as PCR.

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