Original Article

Modified DNA Extraction Technique for Use in Resource-Limited Settings: Comparison of Salting Out Methods versus QIAamp Blood Mini Kit

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Abstract

Background: It is essential that methods for genomic DNA extraction techniques produce high yield and purified DNA. Commercially available DNA extraction kits have taken over the traditional DNA extraction techniques. However, to meet the demands of cost-effectiveness, ready availability, safety, reliability and purity in resource-limited settings, an improved traditional DNA extraction method which meet the above criteria is required. **Aim**: We therefore evaluated the modified salting out and double salt precipitation method, against QIAamp Blood Mini Kit. **Materials and Methods:** In a cross-sectional study, DNA was extracted from venous blood of 60 suspected typhoid fever patients who visited the Komfo Anokye Teaching Hospital diagnostics department to do laboratory investigations that required blood collection. Their DNA was extracted using the three different methods. Spectrophotometric measurement of the yields (ng/µl) and purities (260/280 nm) of the extracted DNA was done. PCR analysis was performed on the DNA extracts to evaluate suitability for downstream analysis. We employed the Mann-Whitney, Kruskal-Wallis and Bland-Altman plots for statistical comparisons. **Results:** The modified double salt precipitation and enzymatic salt precipitation methods produced a higher yield than the QIAamp Blood Mini Kit method (P<0.01 each). The yield from the double salt precipitation method was higher than that of the enzymatic salt precipitation method (P=0.04). The level of purity of DNA extracted from all three methods were comparable (P=0.24). **Conclusion:** Our modified double salt and enzymatic salt precipitation techniques offer higher DNA yields than the commercially available QIAamp Blood Mini Kit and with comparable purity. We recommend the use of these modified techniques in resource-limited settings.

Keywords: Double salt precipitation, DNA extraction, Enzymatic salt precipitation, Modified, QIAamp blood mini kit

Introduction

Elucidation of the Deoxyribonucleic Acid (DNA) structure by Crick and Watson in the 1950's did not only unravel the cellular replicative nature of DNA but also set in motion *in vitro* amplification of DNA. In recent times, DNA technology, genomic analysis and genetic testing are at the heart of modern diagnostics, biological and biomedical research. These disciplines, most times, involve extraction of genomic material, which are found in almost all cells except red blood cells (RBC's) and platelets.[1] The success of genomic or DNA research and diagnostics greatly depends on the quality and quantity of the extracted genomic or DNA materials.[2]

In 1982, Maniatis, *et al.*[3] first demonstrated the phenol-chloroform method as a standard method of DNA extraction. Years later, many traditional methods were developed including solution-based organic solvent methods,[4] the salting out method,[5] solid phase-based methods[6] and magnetic bead methods.[7-9] Most of these methods are time consuming, may require large amount of blood samples and employs toxic organic solvents such as phenol and chloroform.[10]

In recent years however, commercially available DNA extraction kits have taken over the traditional DNA extraction techniques. Notable among them are the Puregene DNA isolation kit (PG) (Gentra Systems, Inc., Minneapolis, MN, USA), MasterPure DNA purification kit (MP) (Epicentre Technologies, Madison, WI, USA) and the QIAamp Blood Kit (QIA) (Qiagen, Inc., Valencia, CA, USA). The use of commercial kits, requires smaller amounts of blood samples and are less time consuming than existing conventional protocols. However, the amount of

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recovered DNA is highly variable and the kits are expensive, with retail prices 50-fold more costly than methods employing standard reagents.[1,11]

Many resource-limited institutions and laboratories have inadequate or no funding for research, making the use of commercial kits a difficult task. Even with adequate funding, the use of commercial kits become a challenge due to time constraints and technical difficulties involved in shipment of commercial kits to researchers. To encourage the use and application of DNA technology in developing countries-where research is less funded by local government and agencies-it is imperative to improve upon the traditional extraction methods to obtain appreciable quantity and quality of DNA extraction for downstream applications.[12-14] Similarly, small laboratories also need to meet the demands of cost-effectiveness, ready availability, safety, speed, reliability and purity. This study sought to develop an in-house DNA extraction techniques from blood samples, by evaluating modified salting out and double salt precipitation methods compared to the commercially available QIAamp DNA Blood Mini Kits.[15-20]

Methods

Ethical considerations

Approval for this study was obtained from the Committee on Human Research, Publications and Ethics of Kwame Nkrumah University of Science and Technology and the Komfo Anokye Teaching Hospital. Participation was voluntary and written informed consent was obtained from each participant. All clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki.

Study design

This comparative cross-sectional study was carried out on 60 suspected typhoid fever patients who visited the Komfo Anokye Teaching Hospital diagnostics department to do laboratory investigations that required blood collection. The prevalence of typhoid fever in Ghana is 4%. With an estimated population of 4,780,380 in Kumasi (2010 population census), a sample size of 60 will be required to obtain a study power of at least 80%. Four mls of blood was taken from each participant who consented. The study was conducted from December 2014 to March 2015.

Sample collection

Four mls of venous blood sample was drawn from each participant and transferred into ethylene diamine tetraacetic acid (EDTA) tubes (BD diagnostic company, NJ, USA), stored at 4°C until assayed. DNA was extracted from each sample using three different methods: a modified double salt precipitation method, a modified enzymatic salting precipitation method, and the standard protocol for the QIAamp Blood Mini Kit.

Enzymatic salt precipitation

The buffers and reagents used for this process were prepared in our laboratory under optimum conditions. To a 2 ml blood sample, 2 mls of Buffer A (0.32 M sucrose, 10 mM Tris HCl, 5 mM $MgCl_2$, 0.75% Triton-X-100, pH 7.6) was added and incubated on ice for 3 minutes. 2 ml of cold sterile deionized water was added, gently mixed (inverted 6 to 8 times) and followed by centrifugation at 3500 rpm for 15 minutes. The supernatant was discarded and the pellet washed by suspension in 2 ml of Buffer A and then in 6 ml of distilled water, followed by centrifugation at 3500 rpm for 15 minutes. The pellet residue was then re-suspended in 5 ml of Buffer B (20 mM Tris-HCl, 4 mM Na_2EDTA, 100 mM NaCl, pH 7.4) and 500 µL of 10% SDS and vortexed vigorously for 30 seconds. 50 µL of Proteinase K solution (1 mg proteinase K, 20 mM Tris-HCl, 4 mM Na_2EDTA and 100 mM NaCl, PH of 7.4) was added, incubated at 55°C for 2 hours. Following Proteinase K digestion, the solution was cooled on ice, thoroughly mixed with a 4 ml of 5.3 M NaCl by vortexing for 15 seconds and pelleted at 4500 rpm for 20 minutes. The recovered supernatant was gently mixed with an equal volume of chilled isopropanol. Precipitated DNA pellets were carefully removed with a sterile Pasteur pipette, transferred into a fresh microcentrifuge tube and washed with 70% ethanol. The DNA was air-dried, resuspended with 300 µL sterile double deionized water and stored at -20°C until ready for use. A negative control which consists of only red blood suspension was added to each batch of extraction.

Double salt precipitation method

To lyse red blood cells (RBC), 900 µl of low salt TKM 1 buffer (10 mM each of Tris HCl-pH 7.6, 10 mM KCl, 10 mM $MgCl_2$, and 2 mM EDTA in 500 ml of distilled water) and 50 µl of 0.75% Triton-X-100 were added to 300 µl of blood in an autoclaved 1.5 ml Eppendorf tube. The mixture was incubated at 37°C for 5 minutes, followed by centrifugation at 8000 rpm for 3 minutes and the supernatant discarded. This was repeated 2-3 times with decreasing amounts of 0.75% Triton-X-100 to completely lyse all the RBCs. The pellets of white blood cell (WBC) were lysed by adding 300 µl of high salt TKM 2 buffer (10 mM each of Tris HCl- pH 7.6, 10 mM KCl, 10 mM $MgCl_2$ 2 mM EDTA, and 0.4 M NaCl in100 ml of distilled water) and 40 µl of 10% SDS and the mixture was incubated at 37°C for 5 minutes. Following incubation, 100 µl of 6M NaCl was added and vortexed to precipitate the proteins. The mixture was clarified at 8000 rpm for 5 minutes and the supernatant was transferred into a new Eppendorf tube containing 300 µl of isopropanol. DNA was precipitated out by slowly inverting the tube and centrifuging at 8000 rpm for 10 mins. DNA pellets were washed with 70% ethanol, centrifuged at 8000 rpm for 5 minutes. DNA was air-dried and resuspended in 50 µl of TE buffer (10 mM Tris HCl-pH 8.0, and 1 mM EDTA). A negative control which consists of only red blood suspension was added to each batch of extraction.

Commercial QIAamp Blood Mini Kit

The QIAamp Mini Kit was designed for rapid purification of an average of 6 to 8 µg of total DNA from 200 µL of whole blood. Extraction of DNA from blood using QIAamp Mini kit (Qiagen Inc., Valencia, CA, USA) was performed according to the manufacturer's instruction. In brief, 200 µL of whole blood was incubated at 56°C with proteinase K and 200 µL Buffer AL for 15 minutes. 200 µL of 95% ethanol was added, transferred into a QIAamp spin column, centrifuged and the supernatant discarded. The column was washed once with Buffer AW1 and then with Buffer AW2. The DNA was eluted in 200 µL of Buffer AE.

Analysis of genomic DNA

Spectrophotometric measurement of the yields and purities of the DNA extracted by the enzymatic salt precipitation, double salt precipitation and the QIAamp Mini Kit methods were done with the NanoDrop ND-1000 Spectrophotometer (Thermo Scientific). The yields were estimated in ng/µl. DNA purity was assessed using the absorbance ratio at 260/280 nm. Values between 1.7 and 2.3 were considered pure. DNA was isolated from the blood of 60 suspected *Salmonella typhi* patients. Both culture positive and negative samples were used. The isolated DNA was further analyzed by conventional polymerase chain reaction (PCR) analysis using Ld1 primer pairs designed to amplify 344 base pairs of org C of *Salmonella typhi*.[20-23] 1 µg of DNA from the three extraction methods in a 50 µl total reaction volume. The Ld1 amplicons were electrophoresed on a 2% Agarose gel, stained with ethidium bromide fluorescent dye and examined under transilluminator (ultraviolet lightbox).

Statistical analysis

Summary statistics values were expressed as median (IQR). We employed the Mann-Whitney, Kruskal-Wallis and Bland-Altman plots for statistical comparisons. A P value of <0.05 was considered significant.

Results

The double salt precipitation and enzymatic salt precipitation methods produced a higher yield than the QIAamp Mini Kit method (P<0.01). The yield from the double salt precipitation method was higher than that from the enzymatic salt precipitation method (P=0.04). The level of purity of extracts from all three methods was comparable [Table 1].

Figure 1 demonstrate via Kruskal-Wallis analysis, that the three methods produced significantly different yields of DNA extracts (P<0.01) all of which had comparable purity (P=0.24).

The QIAamp Blood Mini kit produced extracts with 100% (60/60) purity, the double salt precipitation method, 97% (58/60), and the enzymatic salt precipitation method, 73.7% (44/60) [Figure 2].

To compare the two other methods with the QIAamp Mini kit, we used a Bland-Altman plot. Purity of the QIAamp Mini kit was higher than the double salt precipitation and enzymatic salt precipitation methods. The QIAamp Mini kit however gave a lower yield of DNA compared with the other two methods [Figure 3]. After PCR, a good amplification product was detected by the three methods and this is shown in Figure 4.

Discussion

The quality and yield of DNA (from DNA extraction procedures) are extremely essential in molecular biological experimentations such as PCR, molecular cloning and others. Several protocols are currently available for extracting genomic DNA from biological samples ranging from traditional phenol-chloroform

Table 1: Comparison of DNA yield and purity from the three methods

Parameter	Double salt precipitation	Enzymatic salt precipitation	QIAamp Mini Kit	P value
Yield (ng/uL) median (IQR)	122.15 (89.70-189.00) ****	86.63 (50.10-118.45) ¶¶¶¶	7.40 (4.60-10.60)	0.0417
Purity (A260/A280 nm)	1.80 (1.77-1.83)	1.77 (1.76-1.80)	1.79 (1.73-1.88)	0.0580

Double salt precipitation vrs QIAamp Mini Kit; ¶ Enzymatic salt precipitation vrs QIAamp Mini Kit; P value = Double salt vrs Enzymatic salt precipitation. ****/¶¶¶¶ Significant at <0.0001 level

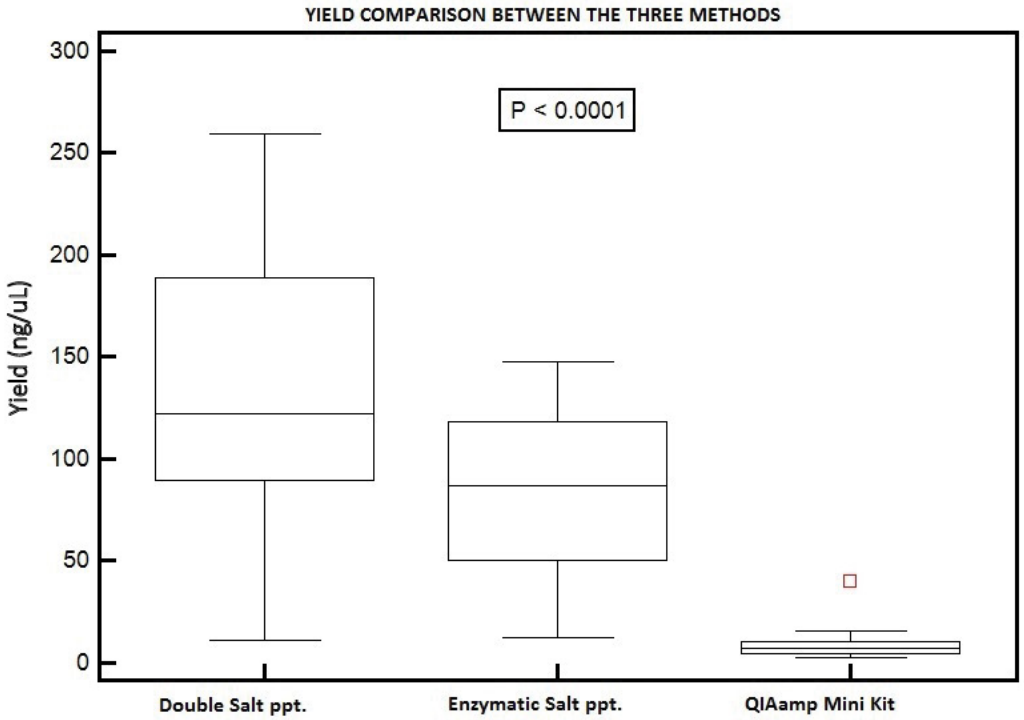

Figure 1: Box and whisker plot of DNA yields based on the three different methods. ppt=precipitation.

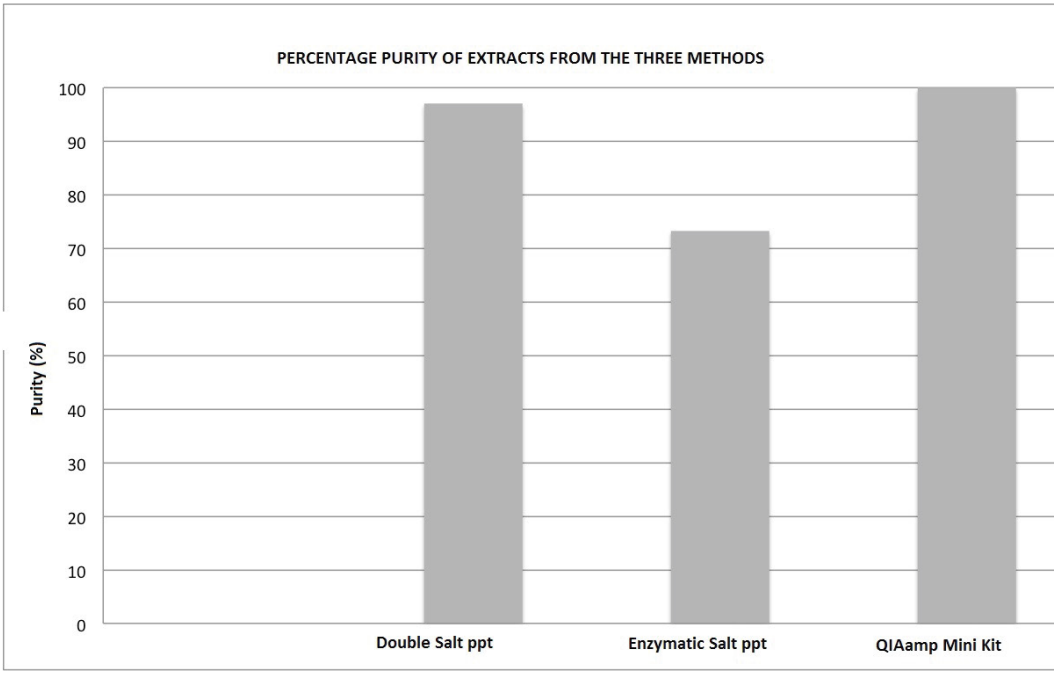

Figure 2: Bar chart showing percentage purities of DNA extracts based on the three methods. ppt=precipitation.

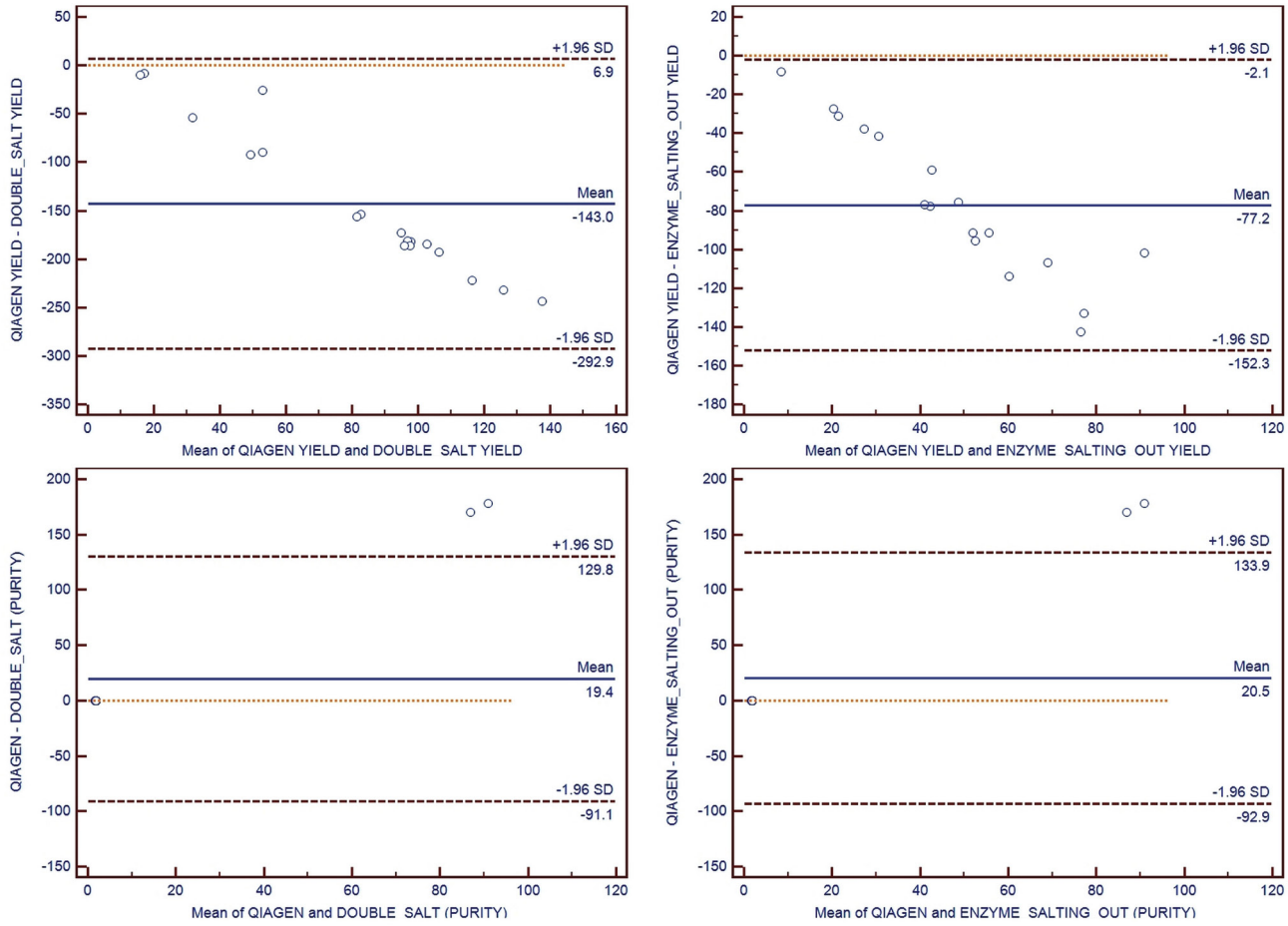

Figure 3: Bland-Altman plots of the standard QIAamp Mini Kit against other two methods. (A) Yield of QIAamp Mini Kit against the double salt precipitation method. (B) Yield of QIAamp Mini Kit against the enzymatic salt precipitation method. (C) Purity of QIAamp Mini Kit against the double salt precipitation method. (D) Purity of QIAamp Mini Kit method against the enzymatic salt precipitation method. Qiagen=QIAamp Mini Kit.

method to commercial kits. The ideal DNA extraction protocol or procedure is tightly intertwined with cost, safety, yield and quality of the DNA. The methods described in this study include significant improvements over existing traditional methods. Firstly, most involve the digestion of cell lysate over night at 37°C with 0.2 ml of 10% SDS, however, our methods do not require overnight digestion. Our method thus reduces turnaround time significantly. Again, most traditional method

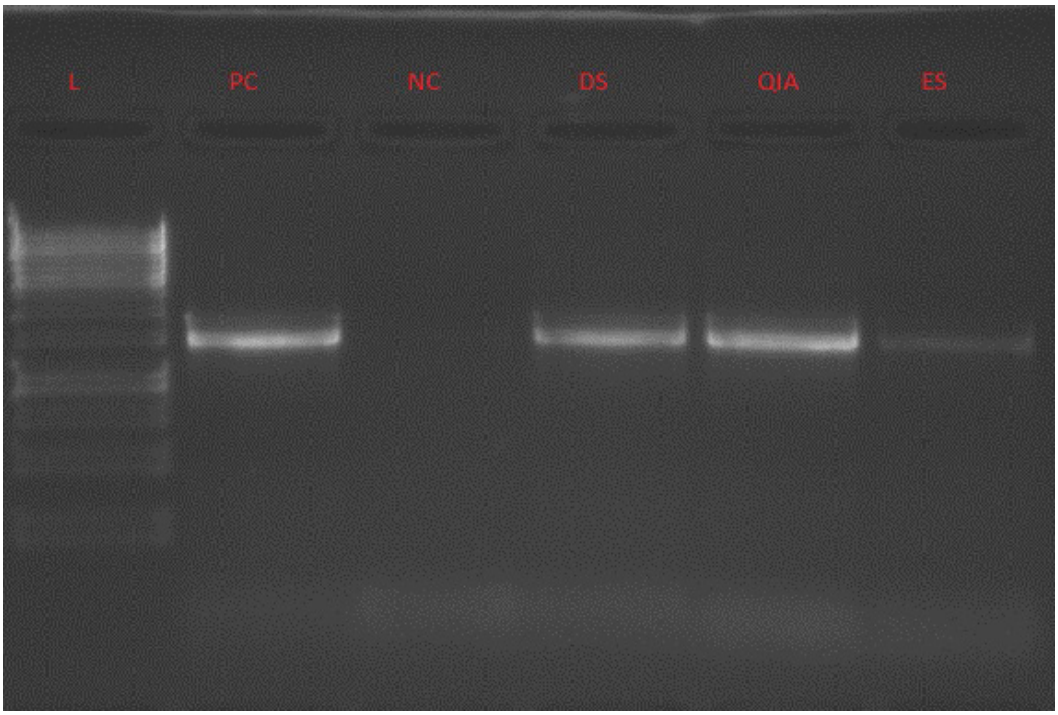

Figure 4: Optimization of extracted DNA from the three methods Agarose gel electrophoresis of Ld1 amplicons of extracted DNA. Gel consists of 1ug of DNA from the three extraction methods in a 50 µl total reaction. From left to right: Lane 1: Ladder; Lane 2: PC-positive control (consist of known DNA from salmonella Typhi); Lane3: NC negative control (consist of red blood cell suspension only); Lane 4: DS-double salt; Lane 5: Q-Qiaamp; Lane 6: ES-enzymatic salt.

employs proteinase K for digestion of proteins and salting out for the removal of proteins whereas our double salting out method do not use proteinase K for removal of proteins, this make our method the most cost-effective. Moreover, phenol and chloroform are hazardous and carcinogenic, this makes their use in most conventional methods unsafe, as such a search for safer and cheaper protocols in attempts to meet the demands of DNA extraction, especially in resource-limited areas is essential. In recent times, researchers have focused on improving the methods of various commercial kits to save time and cost.[2]

In the current study, we compared three methods (modified enzymatic salt precipitation and double salt precipitation methods, and a standard QIAamp Blood Min Kit protocol) for extracting DNA from blood samples. The protocols of these methods were strictly adhered to in order to achieve optimal DNA yields accordingly. It was found that the yield from the modified double salt precipitation (highest) and the enzymatic salt precipitation were higher than that obtained from the QIAamp Blood Min Kit. Although the purity of DNA extracts from the QIAamp Blood Min Kit was highest, but was comparable to that obtained from the other two methods. The high yield observed among the enzymatic and double salts method may be attributed to the higher volume of blood used, 2 ml (2000 µl) and 300 µl respectively. However, the volume/amount of the blood samples used cannot alone explain the higher yield since the enzymatic salting method in which 2000 µl blood was used did not produce a higher yield than the double salting out method, which used 300 µl. The obvious higher yield in the double salting method is due to separate RBC lysis, followed by subsequent precipitation of the lysed RBC proteins whereas in the other methods, RBC and WBC lysis were done simultaneously releasing several protein molecules that may bind to some DNA, which could have been precipitated out.

Similar to the observation in our study, Radheshyam *et al.* recently showed that the QIAamp Blood Mini Kit produced a significantly lower DNA yield than the two other methods (including the salting out).[15] A similar finding was produced by Miller *et al.* and in addition, purity levels were acceptable between the methods.[5] In yet another study, the salt precipitation gave the highest average yield (40.8 µg/mL) and average absorbance ratio (1.90) than the QIAamp Blood Mini Kit (35.3 µg/ml and 1.82 respectively). There was no statistical significance between their yields and purities.[16]

Few studies have showed contrasting results; for example Barbaro *et al.* established that various commercial kits, including the QIAamp Blood Mini Kit, produced higher quantity and quality of DNA extracts than the conventional phenol-chloroform method, using different samples.[17] Studies by Nasiri *et al*. showed that the QIAamp Blood Mini Kit had a higher yield (61.8 µg) and absorbance ratio (2.02), compared to the salt precipitation method. However, there was no statistical difference between the results (p=0.110 and 0.05 respectively). Reasons for the difference could be attributed to the use of laundry detergent in the modified salting out method used in their study.[18] Chacon-Cortes *et al.*[19] compared three DNA extraction protocols, the traditional salting out, a modified salting out and the QIAamp Blood Mini Kit. They found that the QIAamp Blood Mini Kit produced the highest yield and purity but this difference was not statistically significant. The lack of significance in comparing yield and purity of DNA extracts in the above studies may validate the fact that the conventional methods when modified and properly controlled, will be of very good use in DNA extraction from blood, especially in resource-limited settings where there is the need to reduce cost but with high efficiency.

Our modified enzymatic salting out and double salt precipitation techniques do not only offer very high yield of DNA extracts when compared to the commercial kit, but also offer this with in-house prepared reagents, and at a very low cost. This therefore, as compared to other commercial methods may offer higher probability to isolate DNA from blood samples.

It has been suggested that in selecting the ideal methods for DNA extraction, considerations for sensitivity, consistency, speed, and ease of use. Depending on the country of used, it may be important to minimize specialized equipment and pose minimum risk to users. Most importantly, the technique chosen should be able to deliver pure DNA samples ready to be used in downstream molecular applications.[20-22] With comparable purity to the commercially available methods (QIAamp Blood mini Kit), our modified methods can best serve as material for many downstream analyses. To further determine the suitability of the extracted DNA for further downstream analyses, we performed PCR on the isolated DNA from the three methods. As shown in Figure 4, the three methods showed a good amplification product and this demonstrates the excellent performance of the isolated DNA for amplification based diagnostic methodologies.

Conclusion

Our modified double salting out and enzymatic salt precipitation techniques offer higher DNA yields than the commercially available QIAamp Blood Mini Kit with comparable purity to the Our modified methods are suitable for many downstream analyses and are recommended in resource-limited settings.

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