Nig. J. Biotech. Vol. 41(2): 54-63 (Dec. 2024)
ISSN: 0189 1731
Available online at
http://www.ajol.info/index.php/njb/index
and https://bsn.org.ng
DOI: https://dx.doi.org/10.4314/njb.v41i2.5

NIGERIAN JOURNAL OF BIOTECHNOLOGY

Evaluation of optimized DNA Extraction Methods from Bacteria and Whole Blood for Polymerase Chain Reaction

[1]Tahir, M.I., [1]Ahmad, A.E., [2]Waziri, P., [3]Abubakar, S., [2]Wayah, S.B., [2]Tyoapine, D.A., [4]Ahmed, A.D., [3]Sulaiman, M.A.

[1]Department of Medical Laboratory Science, Ahmadu Bello University, Zaria, Nigeria
[2] Department of Biochemistry, Kaduna State University, Kaduna, Nigeria.
[3]Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria
[4] Department of Pharmacology and toxicology, Kaduna State University, Kaduna, Nigeria.

Abstract

The quality of DNA extracted from clinical samples is crucial for the accuracy and reliability of Polymerase Chain Reaction (PCR) in molecular diagnostics. This study compares five optimized DNA extraction methods—Phenol Chloroform (PC), Phenol Chloroform with column (PCC), Boiling (BM), Boiling with Ethanol Precipitation (BME), and a Commercial Kit (CK)—for their efficacy in isolating DNA from bacterial suspension and whole blood from human. The evaluation focused on DNA yield, purity, PCR compatibility, and cost-effectiveness, with a particular emphasis on their use in resource-limited settings. DNA concentration was highest with BM for bacterial samples and with PC for blood samples, though the CK method offered better reproducibility. Significant differences in DNA purity were observed across methods, particularly in *E. coli* and blood samples. PCR amplification was successful for most methods; however, DNA extracted from CK method failed to amplify *E. coli* DNA. Time and cost analyses revealed that while the PC was the most cost-effective, it was also the most time-consuming. Conversely, the CK method was the fastest but most expensive. This study underscores the importance of selecting DNA extraction methods that align with the specific needs of molecular biology applications, balancing cost, time, and performance in resource-limited environments.

Keywords: DNA extraction, Concentration, Purity, Cost-effectiveness
Corresponding author: ghalrash@gmail.com

Introduction

In the realm of molecular biology and diagnostics, the Polymerase Chain Reaction (PCR) has become an indispensable tool for genetic analysis (Sharma et. al., 2024). The accuracy and reliability of PCR critically depends on the quality and quantity of DNA extracted from clinical samples, making the choice of DNA extraction method a pivotal decision. In resource-limited settings, where there are budget constraints and limited access to equipment, the selection of cost-effective and time-efficient DNA extraction methods is of paramount importance (Sakyi et al., 2017).

The objectives of an extraction method are the same regardless of the source or intended use of the DNA: liberating the genetic material from the tissue, fluid or microbe; stabilizing nucleic acids against degradation; eliminating amplification inhibitors; concentrating the nucleic acid material

into a practical volume of an aqueous solution suitable with downstream applications; and standardizing procedures to support precise, sensitive, and repeatable laboratory assays (Boesenberg-Smith et. al., 2012).

While numerous DNA extraction methods exist, there is a conspicuous dearth of studies systematically evaluating their performance with a specific consideration for cost and time efficiency. The impact of method choice on DNA yield, purity, and compatibility with PCR amplification in resource-limited settings has remained insufficiently explored (Beall *et al.*, 2019).

The primary objective of this study is to evaluate and compare five DNA extraction methods—phenol chloroform, phenol-chloroform with column, boiling method, boiling with column method, and a commercially available kit (Zymo Research Quick-DNA™ Miniprep Plus Kit (Cat. No.: D4068) method - with a specific emphasis on their cost-effectiveness and time-efficiency, particularly in resource-limited settings. Through examination using bacterial and whole blood samples, we described the performance attributes of each method and quantified their associated costs and time requirements.

Materials and Methods

Ethical consideration

This research has been designed and conducted with strict adherence to ethical principles, respecting the dignity, rights, safety, and well-being of all human subjects involved. An informed consent was granted by the participants, ensuring confidentiality, and minimizing any potential risks. Ethical approval (No.: NHREC/17/03/2018; via letter with ref: MOH/ADM/744/VOL.1/111004) was obtained from the Health Research Ethics Committee of Ministry of Health, Kaduna State prior to the commencement of the study, in accordance with all applicable national and international guidelines and regulations.

Sample collection, preparation and processing

Bacterial colonies of *S. aureus* and *E. coli* isolated and characterized from patients' urine were sub-cultured in Luria-Bertani (LB) Broth for 14 hours. The bacterial mass was estimated by plate count (serial dilution and colony counting) and reported in cfu/mL. About 20mL of blood sample was collected from a volunteer with a known phenotypic haemoglobin genotype of AA. The absolute white blood cells and red cells count of the blood sample were determined by flow cytometry using Sysmex XP-300™ Haematology Analyser (Sysmex Corporation, Japan).

Ten replicates of each sample (*S. aureus, E. coli* and blood) were subjected to the five DNA extraction protocols. All analyses were conducted at Precision Biomedical laboratory, Kaduna.

DNA Extraction Protocols

i. Phenol-Chloroform Method (PC)

The protocol of Phenol chloroform DNA extraction as initially reported by Sevag et. al., (1938) and Marmur, (1961) were modified in this study. Exactly 200µl of bacterial suspension or blood sample was pipetted into a 2 ml-tube and 400 µl of lysis buffer (Bacterial; 10nM Tris EDTA buffer, 10% SDS, Blood; 10nM Tris EDTA buffer, 10nM KCl, 10nM $MgCl_2$, 0.5M NaCl, 0.5% SDS), 10µl of proteinase-K (20mg/mL) were added into the tube. The tube was incubated at 55°C for 30 minutes and 400µl of equilibrated phenol (pH 7.8) was added, vortexed and centrifuged at 12,000 rpm for 5 minutes. The supernatant was transferred to a fresh tube and 700µl of Chloroform and isoamyl alcohol (24:1) (672 µl Chloroform: 28 µl Isoamyl alcohol) was added and mixed thoroughly, then centrifuged at 12,000 rpm for 5 minutes. The upper layer was transferred to a new tube and 40µl of 3M sodium acetate (pH 5.2) and 400µl absolute ethanol were added and incubated at -20°C for 1 hour. At 4°C, the tube was centrifuged for 15 minutes at 14,000 rpm and the DNA formed a pellet at the bottom of the tube. The supernatant was carefully removed without disturbing the pellet, and 150µl of cold 70% ethanol was added and centrifuged at 14,000 rpm for 2 minutes at 4°C. The supernatant was discarded, and the DNA pellet was allowed to dry at room temperature for 10 minutes. The DNA pellet was resuspended in 100µl of Tris EDTA buffer (10Mm Tris HCl, 1mM EDTA) and was briefly centrifuged and stored at -20°C.

ii. Phenol-Chloroform with column Method (PCC)

The Phenol-Chloroform protocol described above was further modified to include elution of the DNA using a spin column. The spin column was introduced in the protocol after the centrifugation at the Chloroform: isoamyl alcohol stage. Here the upper layer was transferred to a new tube and an equal volume of binding buffer (4M guanidine hydrochloride, 1M potassium acetate, pH5.5) was added and mixed gently. The mixture was transferred into the spin column loaded on collection tube and centrifuged at 12,000 rpm for 30 seconds. The column membrane was washed twice by centrifugation at 12,000 rpm for 1 minute using 750µl of 75% ethanol. The column was inserted into a new tube and 200µl Tris EDTA buffer was added and centrifuged at 12,000 rpm for 1 minute to elute the DNA.

iii. Boiling Method (BM)

In boiling method, 200µL of bacterial suspension or blood was transferred into a sterile 2ml-tube and 500µL of sterile nuclease-free water was added. The tube was centrifuged at 10,000 rpm for 5 minutes and the supernatant decanted. The cells were resuspended in 500µL of sterile nuclease-free water and the step repeated. The cells were again resuspended in 500µL of sterile nuclease-free, vortexed and incubated in a heating block at 100°C for 10 minutes. Thereafter, the tube was incubated at -20°C for 7 minutes, then centrifuged at 14,000 rpm for 10 minutes. The resulting supernatant containing the DNA was gently decanted into a new tube.

iv. Boiling Method with Ethanol Precipitation (BME)

The boiling method described above was further modified by adding equal volume of cold absolute ethanol to the supernatant and 40µL of 3M sodium acetate and incubated at -20°C for 10 minutes. The tube was centrifuged at 12,000 rpm for 10 minutes and the supernatant discarded. Exactly 300µL of 70% ethanol was added and centrifuged at 12,000 rpm and the supernatant decanted. The tube was allowed to dry at room temperature for 10 minutes and resuspended in 100µL Tris EDTA buffer (10Mm Tris HCl, 1mM EDTA).

v. Commercial Kit

Zymo Research Quick-DNA™ Miniprep Plus Kit (Cat. No.: D4068), a commercial kit for DNA extraction from different biological samples was employed as the standard and the manufacturer's instruction was adopted to extract DNA from 200µL of the bacterial suspension and blood samples.

Estimated Time and Cost Implication

The time taken from the beginning to the final stage of DNA recovery for each procedure was noted. The average cost of materials for each procedure was also noted and compared.

Estimation of DNA Concentration and Purity

The concentration and purity of the extracted DNA from the five methods were determined using NanoDrop (ThermoFisher Scientific, Waltham, USA) Spectrophotometer. The mean and standard error mean of concentration and purity of extracted DNA were determined. One-way ANOVA was used to determine significant difference of concentration and purity among the five protocols using GraphPad Prism version 8.

Amplification of 16s rRNA and normal β-globin genes

Extracted bacterial DNA for both *S. aureus* and *E. coli* from each method were subjected to PCR to detect 16s rRNA portion of the genome using 27F and 1492R primers as presented in Table 1. A 20 µl reaction was performed and 10µl of Taq Mix (Bioer Technology, China) was placed into a PCR strip and 1µl each of forward primer (27F) and reverse primer (1492R) were added. Five microliter (5 µl) of extracted bacterial DNA and 3 µl of nuclease free water were also added to the mix. The mixture was placed in PTC100™ thermocycler (MJ Research, Inc., USA). The cycling conditions for the PCR as optimized were 95°C for 1 minute then 40 cycles of 94° C for 30 seconds, 52° C for 40 seconds, 72° C for 1 minutes and final extension at 72°C for 5 minutes.

For the amplification of normal β-globin gene, extracted blood DNA from each method was subjected to the PCR. The PCR mix for amplification of the normal β-globin gene was prepared as the preceding protocol with WT-AS

and WT-CP517 primers (Table 1) and DNA extracted from blood sample. The cycling conditions for the PCR as optimized were 95˚C for 3 minutes then 40 cycles of 94˚ C for 60 seconds, 52˚C for 60 seconds, 72˚ C for 60 seconds and final extension at 72˚C for 7 minutes.

Table 1: Primers sequences for the amplification of bacterial 16s rRNA and human normal β-globin gene genes

Region of interest	Primer Name	Sequence	Amplicon size	Reference
16s rRNA	27F	5′-AGAGTTTGATCCTGGCTCAG-3′	1500 bp	(Raji et. al., 2008)
	1492R	5′-GGTTACCTTGTTACGACTT-3′		
Normal β-globin gene	WT-AS	5′-ATGGTGCACCTGACTCCTGA-3	517 bp	(Waterfall & Cobb, 2001)
	WT-CP517	5′-CCCCTTCCTATGACATGAACT-3′		

Agarose Gel electrophoresis

The PCR products were analyzed by agarose gel electrophoresis, employing a Serva DNA stain G (Serva, Germany) to stain the agarose gel. The stained gel was submerged in Tris Acetate EDTA (TAE) buffer and electrophoresed at 120 volts for 30 minutes. The gel was subjected to UV light exposure for visualization, utilizing a gel documentation system (Gel Doc 2000, Bio-Rad Laboratories, Inc., USA). To ascertain the sizes of the PCR amplicons, a 100bp DNA ladder (TransGen Biotech Co Ltd, China) was concurrently loaded onto the gel alongside the PCR products.

Results

Samples characteristics

The cell densities of *S. aureus* and *E. coli* in LB broth were 4.3 x 10^9 cfu/mL and 7.5 x 10^9 cfu/mL respectively. The absolute counts of white blood cells (WBC) and erythrocytes in the blood sample were 4.76 x 10^9/L and 3.86 x 10^{12}/L respectively.

DNA Concentration and Purity

Comparison of the DNA extraction methods showed significant difference in the DNA yield across the three samples Table 2). Boiling protocol had the highest DNA concentration in the bacterial samples (*S. aureus and E. coli*) while phenol-chloroform protocol had highest DNA concentration in the blood sample. The variations in DNA concentration for *S. aureus*, *E. coli* and blood samples across the methods were presented in Table 2. There was significant difference in DNA concentration extracted from *S. aureus* between BM and PCC ($p=0.003$), PC ($p=0.0078$), BME ($p=0.0002$) and Kit ($p<0.0001$). There was also significant difference in DNA concentration extracted from *E. coli* between BM and PCC ($p=0.0053$), BMC ($p=0.0002$) and Kit ($p<0.0001$). Significant difference was also observed between PC and PCC (0.0394), BME (0.0023), Kit ($p<0.0001$). The same pattern was observed in BME and Kit ($p=0.0002$). There was a significant difference in DNA concentration extracted from blood between BMC and BM ($p=0.0070$) and Kit ($p=0.0167$). There was also significant difference between PC and Kit ($p<0.0001$). An important quality exhibited by the Kit protocol is better reproducibility in the extracted DNA concentration.

There was no significant difference in DNA purity among the compared protocols in *S. aureus* samples (Table 3). Better DNA purity was observed in PCC for all the sample types. There was a significant difference in purity of DNA

extracted from *E. coli* using compared protocols. There were significant variations in purity of DNA extracted from E coli between PCC and BMC (p=0.0318) and Kit (p=0.0047). In the same vein, there was also significant variation between PC and BME (0.0021). There was a significant difference in purity of DNA extracted from blood among the protocols. The purity of DNA extracted by PCC had significant difference with that of PC (p=0.0422) and BM (p=0.0024). In the same vein, BM and Kit (p=0.0158) had significant difference in purity.

Table 2: Mean Concentration of extracted DNA from different samples using different protocols

Sample	Phenol Chloroform (µg/mL) (±SEM)	Phenol Chloroform with column (µg/mL) (±SEM)	Boiling (µg/mL) (±SEM)	Boiling with Ethanol Precipitation (µg/mL) (±SEM)	Commercial Kit (µg/mL) (±SEM)	p-value*
S. aureus	63.6 ± 6.3	52.3 ± 7.1	101.4 ± 8.5	51.5 ± 9.2	34.6 ± 9.1	0.0001
E. coli	47.3 ± 4.4	31.3 ± 5.5	51.4 ± 2.7	25.8 ± 4.2	7.7 ± 0.9	0.0001
Blood	69.3 ± 3.9	46.7 ± 10.1	36.3 ± 2.5	49.1 ± 10.1	21.2 ± 1.7	0.002

*Determined by one way ANOVA.

Table 3: Mean Purity of extracted DNA from different protocols and sample type

Sample	Phenol Chloroform (260/280) (±SEM)	Phenol Chloroform with column (260/280) (±SEM)	Boiling (260/280) (±SEM)	Boiling with Ethanol Precipitation (260/280) (±SEM)	Commercial Kit (260/280) (±SEM)	p-value*
S. aureus	1.4 ± 0.2	1.3 ± 0.1	1.1 ± 0.03	1.2 ± 0.1	1.2 ± 0.2	0.136
E. coli	1.8 ± 0.02	1.7 ± 0.1	1.6 ± 0.04	1.4 ± 0.1	1.4 ± 0.04	0.001
Blood	1.6 ± 0.1	1.4 ± 0.1	1.3 ± 0.03	1.4 ± 0.1	1.5 ± 0.1	0.003

*Determined by one-way ANOVA.

PCR

The expected amplicons of 1500bp were obtained in 9 out of the 10 bacterial DNA samples from the 5 methods (2 from each method) using the 27F and 1492R primer sequences. There was no amplification from the *E. coli* sample extracted by the Kit method (Figure 1). Five DNA samples extracted from blood using the 5 methods were amplified using the WT-AS and WT-CP517 primer sequences and produced 517bp expected amplicons (Figure 2).

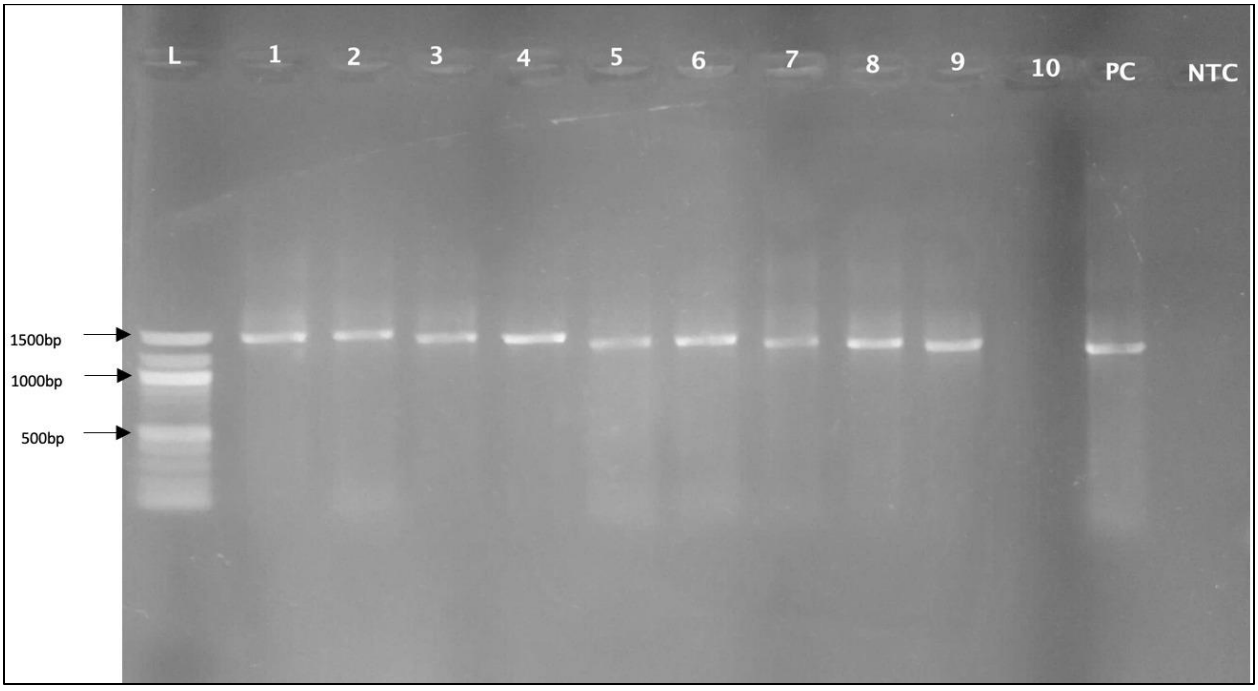

Fig 1: Gel Electrophoretogram of PCR Products of 16s rRNA of the selected Samples.

1 (*S. aureus*) & 2 (*E. coli*) with DNA from phenol-chloroform; 3 (*S. aureus*) & 4 (*E. coli*) with DNA from phenol-chloroform with column; 5 (*S. aureus*) & 6 (*E. coli*) with DNA from boiling; 7 (*S. aureus*) & 8 (*E. coli*) with DNA from boiling with ethanol; 9 (*S. aureus*) & 10 (*E. coli*) with DNA from commercial kit; L: Ladder; PC: Positive Control; NTC: No Template Control.

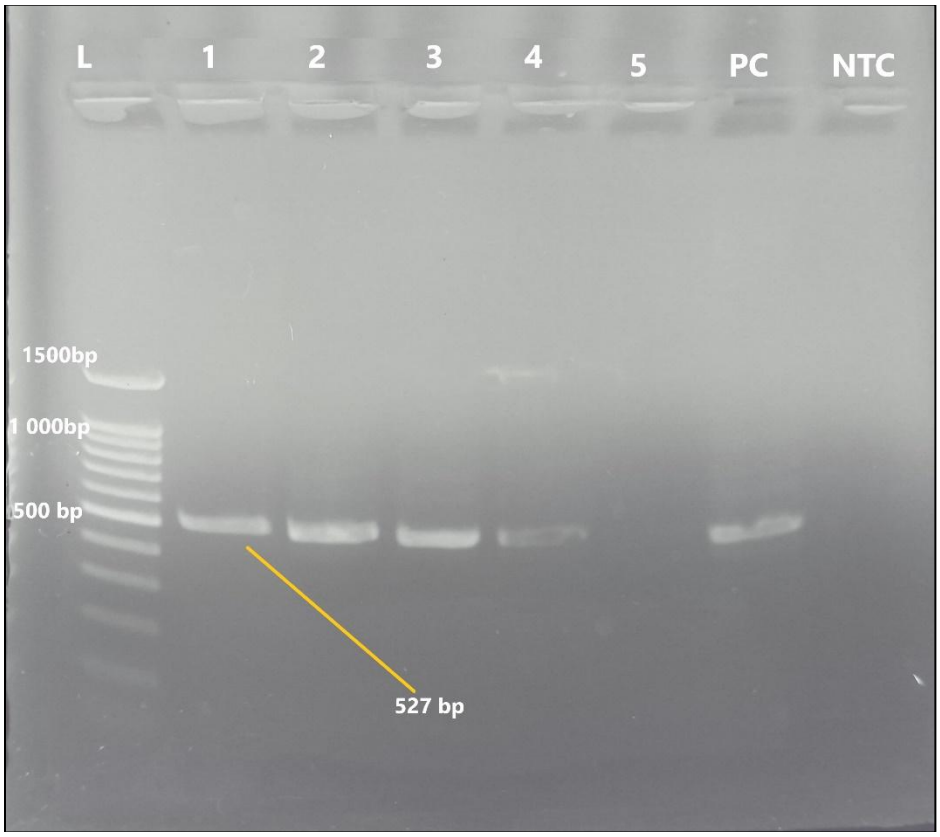

Fig 2: Gel Electrophoretogram of PCR Products of the region of Normal β-globin gene extracted from blood Samples.

(1) DNA from phenol chloroform; (2) DNA from phenol chloroform with column; (3) DNA from boiling; (4) DNA from boiling with ethanol; (5) DNA from the commercial kit; L: Ladder; PC: Positive Control. NTC: No Template Control.

Cost and Time Considerations

Table 3 presents a comparative analysis of five different DNA extraction methods based on two crucial factors: time taken to extract and cost per sample. Phenol Chloroform extraction was the most time-consuming, taking 127 minutes, but offers a relatively lower cost at 0.8 USD per sample. In contrast, the application of column in Phenol Chloroform significantly reduced the extraction time to 43 minutes but with a slightly higher cost of 1.05 USD per sample. Boiling and boiling with ethanol precipitation required 37 and 67 minutes, respectively, with costs of 0.25 USD and 0.5 USD per sample. On the other hand, utilizing a Commercial Kit is the quickest method, taking only 10 minutes, but it comes with a higher cost of 1.62 USD per sample.

Table 3: Cost and Time Considerations in DNA Extraction Method Selection

Extraction Method	Time taken (Minutes)	Cost per sample, ₦(USD)*
Phenol-chloroform	127	800 (0.8)
Phenol-chloroform with column	43	1,050 (1.05)
Boiling	37	250 (0.25)
Boiling with ethanol precipitation	67	500 (0.5)
Commercial Kit	10	6000 (6.0)

*₦1,000/$ (as at Nov. 2023).

Discussion

This study demonstrated variations in DNA concentration and purity across different extraction methods. The results of the study provide important insights into the selection of appropriate DNA extraction methods for bacterial and blood samples. DNA extraction is a critical step in many biological and clinical research applications, and the choice of method can significantly influence the quality and quantity of DNA obtained. Large-scale molecular investigations require the choice of a suitable technique for the quick, safe, and cost-effective isolation of pure DNA (Boesenberg-Smith et al., 2012).

DNA Concentration

The differences in DNA yield observed across the methods could have stemmed from variations in lysis efficiency, where some methods may not fully break down cells, leaving DNA trapped. Additionally, differences in DNA binding and recovery during extraction can lead to incomplete DNA retrieval. The observed variations in DNA concentration among the extraction methods have several implications and it directly affects downstream applications. In polymerase chain reaction (PCR) and DNA sequencing, the concentration of DNA template affects the efficiency and success of these reactions. Low DNA concentration can result in poor amplification or sequencing results (Ali et. al., 2017). Higher DNA concentrations allow researchers to split samples for multiple experiments or store them for future use, reducing the need for repeated DNA extraction. Some assays and diagnostic tests with low sensitivity may require specific DNA concentrations to achieve the required sensitivity and detection limits (Lucena-Aguilar et. al., 2016).

While the kit protocol yielded less DNA compared to the other methods, it provided precise measurement and could potentially provide an advantage in the context of molecular diagnosis, prognosis, and disease monitoring within a medical laboratory setting.
Although the phenol chloroform method is thought to be a historical standard method and produced higher DNA concentration in our investigation, its frequently laborious and manual nature makes it unsuitable for application in clinical laboratory settings. Several transfer processes may result in contamination and possible phenol exposure (Boesenberg-Smith et al., 2012).

DNA Purity

DNA purity is influenced by the co-extraction of contaminants like proteins, salts, and inhibitors, which varies with the reagents and protocols used. Furthermore, the effectiveness of washing and separation steps, including removal of residual chemicals or organic solvents, significantly impacts purity levels. DNA purity is crucial for various molecular biology applications. Impure DNA can lead to contamination and suboptimal results in downstream analyses, such as PCR, sequencing, or gene expression studies. Contaminants, such as proteins, RNA, or other impurities, can interfere with downstream applications. Impure DNA can inhibit the activity of enzymes used in various molecular biology techniques (Sidstedt et al., 2018). Purity ensures that the DNA sample is free from such contaminants Our findings indicate that Phenol Chloroform method consistently provided DNA with better purity across all sample types. However, the protocol involve use of phenol and it is not time efficient.

Our study indicated apparent higher purities in *E. coli* than in *S. aureus.* However, in a similar study with enhanced protocols for phenol chloroform and boiling DNA extraction methods from methicillin-resistant *Staphylococcus aureus* (*MRSA*), the mean purities of 1.77 ± 0.03 and 1.22 ± 0.01 were reported respectively (Ahmed et al., 2014). The same study reported purities of 1.55 ± 0.01 and 1.17 ± 0.03 from Gram-negative ESBL producer for phenol chloroform and boiling respectively.

PCR Amplification

The success of PCR amplification is a critical factor for many molecular biology experiments. In our study, most methods demonstrated high PCR success rates, with the expected amplicons obtained in most samples. However, it is worth noting that the Commercial Kit method failed to yield amplification in *E. coli* samples. This finding emphasizes the necessity of verifying the compatibility of chosen DNA extraction methods with downstream applications.

Cost and Time Considerations

The choice of a DNA extraction method should also consider practical considerations, such as cost and time. Researchers with time constraints may opt for faster methods like the Commercial Kit, despite its higher cost per sample. On the other hand, projects with more extensive sample sizes and lower budgets may prefer the cost-efficient Phenol Chloroform method, even though it is more time-consuming. The choice of extraction method should thus consider the trade-off between time efficiency and cost-effectiveness, with Phenol Chloroform being the most time-consuming but cost-efficient option, while the Commercial Kit offers speed at a higher cost per sample.

In conclusion, the selection of a DNA extraction method is not a one-size-fits-all decision. Researchers and professionals must cautiously weigh the trade-offs between DNA yield, purity, reproducibility, cost, and time efficiency. By understanding the implications of each extraction method, researchers can make informed choices that bring into line with the objectives of their studies or areas of application. Standardization and careful consideration of these factors are crucial for preserving the integrity and reproducibility of scientific research.

References

Ahmed, O. B., Asghar, A. H., & Elhassan, M. M. (2014). Comparison of three DNA extraction methods for polymerase chain reaction (PCR) analysis of bacterial genomic DNA. African Journal of Microbiology Research, 8(6), 598-602.

Ali, N., Rampazzo, R. d. C. P., Costa, A. D. T., & Krieger, M. A. (2017). Current nucleic acid

extraction methods and their implications to point-of-care diagnostics. BioMed research international, 2017(1), 9306564.

Beall, S. G., Cantera, J., Diaz, M. H., Winchell, J. M., Lillis, L., White, H., ... & Boyle, D. S. (2019). Performance and workflow assessment of six nucleic acid extraction technologies for use in resource limited settings. *PLoS One*, *14*(4), e0215753.

Boesenberg-Smith, K. A., Pessarakli, M. M., & Wolk, D. M. (2012). Assessment of DNA yield and purity: an overlooked de tail of PCR troubleshooting. *Clinical Microbiology Newsletter*, *34*(1), 1-6.

Lucena-Aguilar, G., Sánchez-López, A. M., Barberán-Aceituno, C., Carrillo-Avila, J. A., López-Guerrero, J. A., & Aguilar-Quesada, R. (2016). DNA source selection for downstream applications based on DNA quality indicators analysis. *Biopreservation and biobanking*, *14*(4), 264-270.

Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from micro-organisms. *Journal of molecular biology*, *3*(2), 208-IN201.
Raji, A. I., Möller, C., Litthauer, D., van Heerden, E., & Piater, L. A. (2008). Bacterial diversity of biofilm samples from deep mines in South Africa. *Biokemistri*, *20*(2).

Sevag, M., Lackman, D. B., & Smolens, J. (1938). The Isolation of the Components of Streptoeoeeal Nueleoproteins in serologieally Active Form.

Sharma, P., Mishra, A., Misra, S., Gondhali, U., & Chauhan, T. (2024). Polymerase Chain Reaction: An Indispensable Molecular Biology Tool of 21st Century. In *Fundamentals of Forensic Biology* (pp. 209-226). Springer.

Sidstedt, M., Hedman, J., Romsos, E. L., Waitara, L., Wadsö, L., Steffen, C. R., ... & Rådström, P. (2018). Inhibition mechanisms of hemoglobin, immunoglobulin G, and whole blood in digital and real-time PCR. *Analytical and bioanalytical chemistry*, *410*, 2569-2583.

Waterfall, C. M., & Cobb, B. D. (2001). Single tube genotyping of sickle cell anaemia using PCR-based SNP analysis. Nucleic acids research, 29(23), e119-e119.

Sakyi, S. A., Kumi, B., Ephraim, R. D., Danquah, K. O., Osakunor, D., & Baidoe-Ansah, D. (2017). Modified DNA extraction technique for use in resource-limited settings: comparison of salting out methods versus QIAamp blood mini kit. Annales of Medical and Health Sciences Research, 7(3).