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Recovery of whole genomic DNA from stored Sickle Cell Disease blood samples by the optimization of standard procedure

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Abstract: *COVID pandemic waves has been resulted in an unspecified lockdown period impacting not only the life and its basic necessities but also hampering biological research settings from basic to advanced scale of works. Blood samples are the sought-after sources for various biochemical and genomic extraction procedure across clinical settings providing significant insights of any underlying disease. Long-term storage of blood samples has been reported to have influence over the yield and the quality of DNA. Along with storage conditions, studies have revealed the influence of intrinsic factors namely temperature, pH, genotoxic agents, alkylating agents, disease state and cell type on genomic extraction. In the current study, we are reporting a data on the isolation of genomic DNA from 2 months old SCD whole blood samples happened to be stored during pandemic lockdown, by the optimization of standard protocols at appropriate steps, successfully recovering good yield of DNA.*

Key Words: *Disease samples, Genomic DNA extraction, Storage, Lysis, Quantification.*

1. INTRODUCTION:

COVID pandemic waves has resulted in an unspecified lockdown period impacting not only the life and its basic necessities but also has hampering biological research settings from basic to advanced scale of works such as maintenance of study animals, instrumentations, sample collections, storage of biological samples, specimens etc. Blood samples are the sought-after sources for various biochemical and genomic extraction procedure across clinical settings providing significant insights of any underlying disease in question. Long-term storage of blood samples have been reported to have influence over the yield and the quality of DNA along with the contamination issues.[3] Previous studies have cited the interference of intrinsic factors such as temperature, pH, genotoxic agents, alkylating agents, disease state and cell type on overall yield of extracted DNA.[5] Reports have shown the elimination of the necessity of -80°C storage, but also have obtained high quality DNA from microscale volume of samples stored at 4°C.[4, 5, 10] Various authors have revealed fine quantity, quality and intact DNA can be obtained from blood samples stored at 4°C.[22, 24, 25] Storage of blood samples at 4°C with different incubation period as alternative source has been reported elsewhere.[21, 23] In the current study, we are reporting a data on the isolation of genomic DNA from 2 months old SCD whole blood samples happened to be stored during pandemic lockdown, by the optimization of standard protocols at appropriate steps, successfully recovering good yield of DNA.

2. STUDY OBJECTIVE: Recovery of DNA from stored disease blood samples.

3. METHODOLOGY:

Sample collection:

SCD human blood samples of 1 mL from six adults at an average age of 24 (both male and female) along with a normal blood sample from a volunteered healthy individual were collected in EDTA tubes (BD vacutainer) from JSS Hospitals, Mysore, Karnataka, India, stored at 4°C for routine laboratory DNA extraction

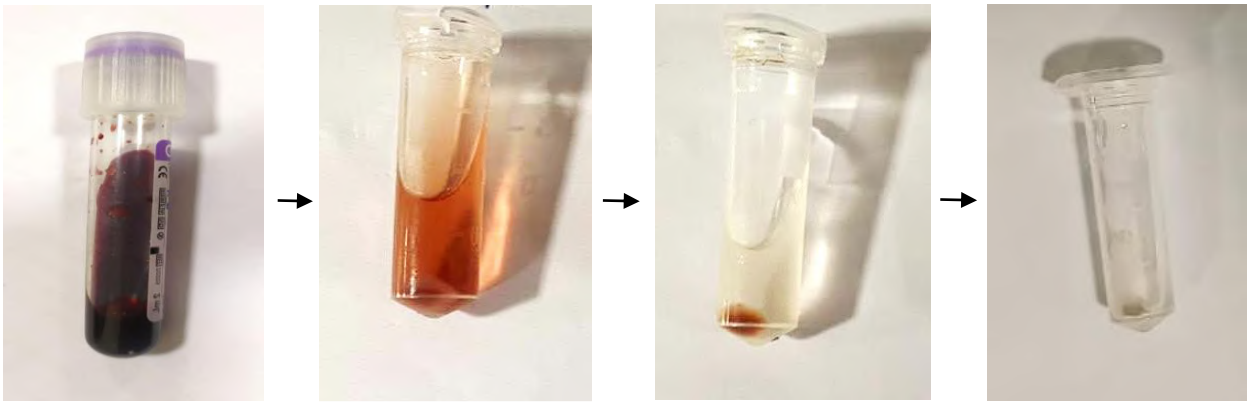
works. However, Untimely interference of COVID pandemic second wave resulted in an unspecified lockdown period happened to continue for 2 months resulting in an increase in the overall storage (4°C) period of previously collected blood samples to 66 days by the time University labs were permitted to function.

RBC Lysis from standard procedure:

More than two months old (66 days) stored blood was subjected to RBC lysis according to the standard procedures -1350µL of TKM 1 and 75 µL of 1x triton-X was added to 450 µL of EDTA blood in five autoclaved vials and were incubated at 37°C for 5 mins (fig B). After incubation cells were centrifuged at 8000 rpm for 6 mins at 4°C, supernatant was discarded and a reddish undigested pellet was observed (fig C) to be retained across all the samples and the results being further discussed.[3, 7, 8, 23, 24]

Optimization:

When extraction process was performed using the standard procedure, lysis was not found to be complete. Incomplete lysis was observed with the minimal amount of pellet formation along with red coloration. To resolve this, 100% triton X was added to aid the cell lysis. To the retained red pellet of SCD samples in five vials from standard procedure, 100% triton-X was added and subjected to six rounds of lysis step with decreasing volume of 100% triton-X to the pellet in the order of 70 µL, 60 µL, 50 µL, 40 µL, 30 µL, 20 µL corresponding to each sample with increasing rounds of centrifugation upto 6 times until RBCs showed complete lysis resulting in a white pellet (Fig D).



A B C D

Figure 1: (A) SCD Sample, (B) Vial after first RBC lysis, (C) Red pellet obtained due to incomplete RBC lysis, (D) White pellet obtained by optimizing the standard protocol.

Cell lysis:

After the said optimization, the completely lysed samples were carry forwarded for further lysis steps according to the standard procedure. To the obtained white pellet from the five samples, 450 µL of TKM 2 buffer and 80 µL of 10% SDS was added to each sample with thorough mixing followed by incubation at 37°C for 5 mins. At the end of incubation, 150 µL of 6 M NaCl solution was added to each vial and vortexed for 20 seconds to wash the remaining proteins, centrifuged at 8000 rpm for 6 mins and the supernatant was collected.[8, 23, 24] The obtained supernatant from five vials were transferred to fresh vials prefilled with 450 µL of isopropanol and the DNA was precipitated by inverting the vials slowly followed by centrifugation at 8000 rpm for 12 mins at 4°C to pellet down the DNA. To the obtained DNA pellet, 70% ethanol was added and vortexed to remove any excess salts, followed by centrifugation at 8000 rpm for 9 mins at 4°C, air-dried at room temperature and suspended in 50 µL of 10 mM TE buffer and stored at 4°C. DNA isolated was subjected to quantification by Nano-drop (DeNovix) and the readings presented.[3, 7]

Digestion of Genomic DNA:

The isolated DNA from the five samples were subjected to restriction digestion using EcoR1. 2 µg of DNA from each sample was incubated with 4 µL of restriction enzyme EcoR1 separately for 1 hour 3 minutes at 37°C. After incubation, the enzyme was heat inactivated at 65°C for 20 minutes.[30] The digested DNA from

the five samples were run on 2.5% agarose gel in the concentration of 2 µg separately. Electrophoresis (Biobee Tech) was performed at 75V and the results were analyzed using gel documentation system (Syngene).

4. RESULTS:

Table 1: Absorbance of stored SCD samples at 260/280 nm

Samples	Standard Protocol OD (260/280)	Optimized Protocol OD (260/280)
1	1.01	1.74
2	1.27	1.66
3	0.63	1.66
4	1.07	1.77
5	0.89	1.74

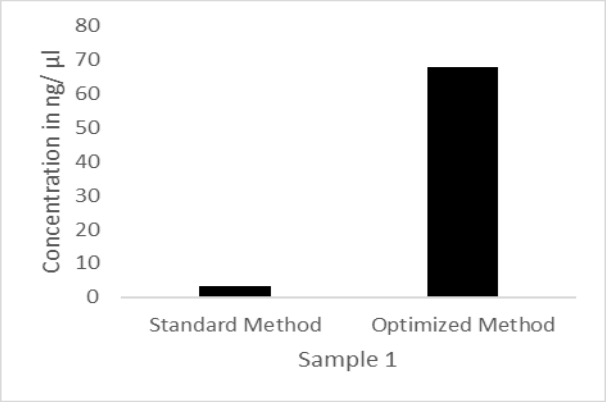


Figure 2 : Comparison of DNA concentration of sample 1 obtained using standard and optimized protocol.

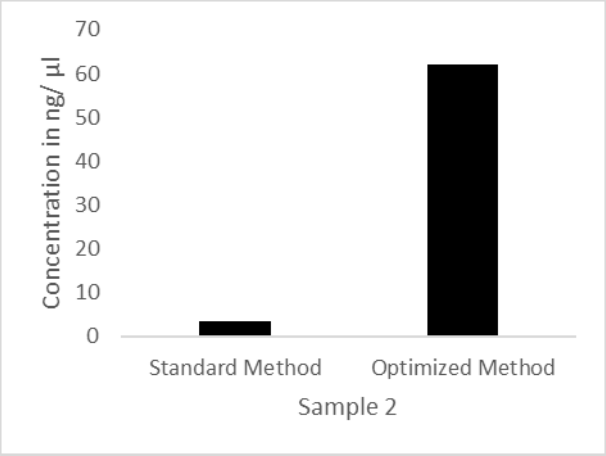


Figure 3: Comparison of DNA concentration of sample 2 obtained using standard and optimized protocol.

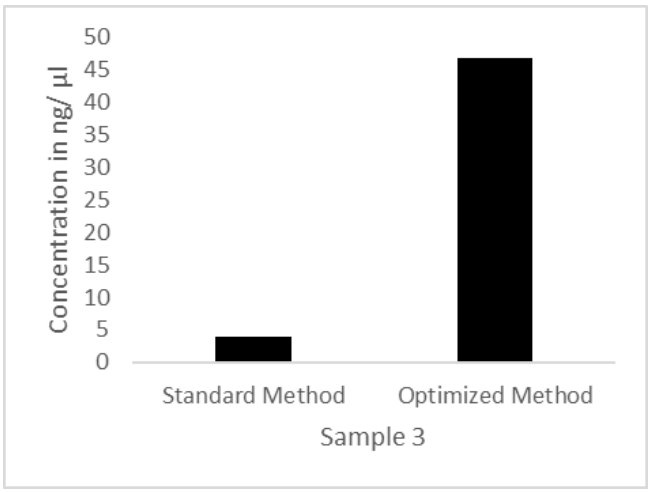


Figure 4: Comparison of DNA concentration of sample 3 obtained using standard and optimized protocol.

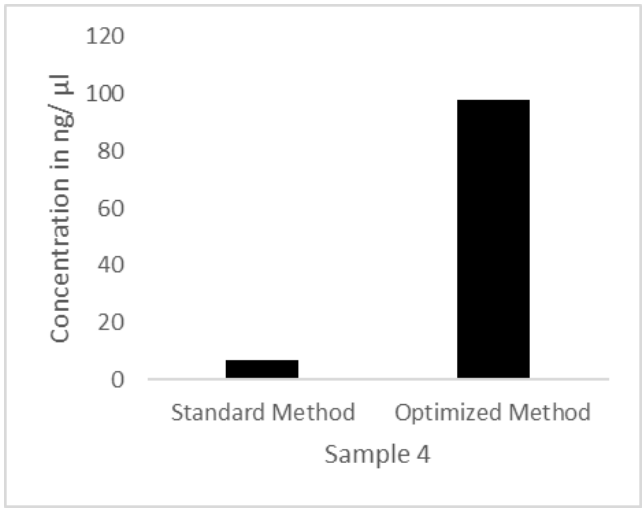


Figure 5: Comparison of DNA concentration of sample 4 obtained using standard and optimized protocol.

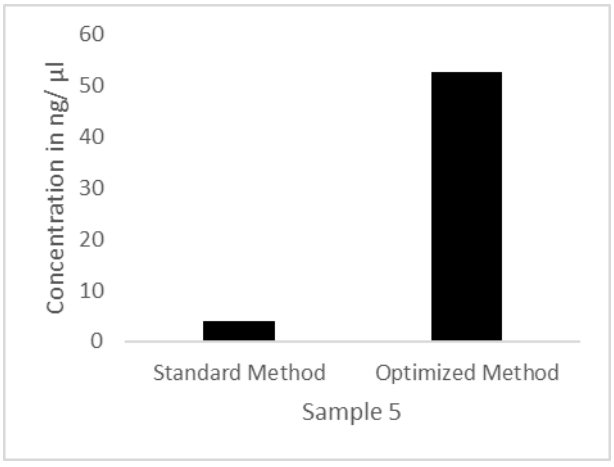


Figure 6: Comparison of DNA concentration of sample 5 obtained using standard and optimized protocol.

Figure 2, 3, 4, 5, 6 depicts the comparison of concentration of DNA obtained in optimized protocol with standard protocol for the long term stored five different SCD human Blood samples.

The obtained quantified results indicated that the yield of DNA was found to be much higher. The DNA so obtained was given an electrophoretic run. After electrophoresis the samples in each lane were compared with a standard DNA ladder. Obtained gel result indicated that the DNA of five different SCD samples were partially digested showing the presence of DNA which were of higher molecular weight as shown in Figure 7.

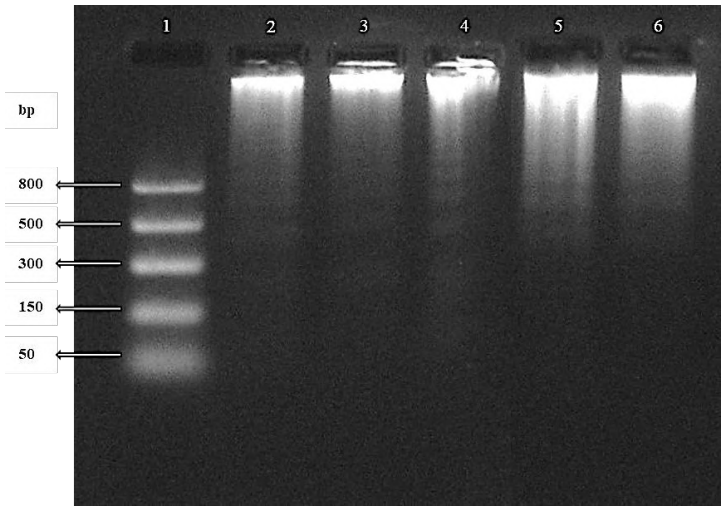


Figure 6: 2.5% Agarose gel electrophoresis of DNA isolated from stored SCD blood samples. Lane 1 - ladder; Lane 2,3,4,5 – samples.

5. DISCUSSION:

Prolonged storage of blood samples causes lysis of erythrocytes which corresponds to the increased haemoglobin content present in the leukocyte pellet and thereby affecting the genomic DNA recovery[6]. The SCD samples procured were stored at 4˚C since research activities were affected to a great extent due to Covid-19 pandemic. As of lockdown measures implemented by the state government, university research labs came to a halt for an unknown period of time resulting in the increase in the storage of collected disease blood samples upto more than two months. All the stored samples were processed post-lockdown following the standard protocol resulting in an incomplete lysis of the sample cells with the minimal amount of pellet formation along with red coloration owing to the presence of RBCs in the pellet. To resolve this, 100% triton-X was added to aid the cell lysis with increasing rounds of centrifugation in order to overcome the difficulties identified during the experimentation with reference to the standard protocol. Human red blood cells can be disintegrated with 100% triton-X which is a non-ionic detergent and also has the ability to lyse RBCs invitro mentioned elsewhere.[29] The protocol has been optimized by adding 100% triton-X to the pellet retained after the RBC lysis and subjecting it to six rounds of lysis step with decreasing volume of 100% triton-X to the pellet in the order of 70 µL, 60 µL, 50 µL, 40 µL, 30 µL, 20 µL to each vial. Also, in agreement with previous studies[8, 28], extraction of DNA with Triton-X has resulted in higher yield of undegraded DNA. On the contrary to the published reports of recovery of less yield of DNA from stored samples, our study was able to recover good amount of genomic DNA. Reports claiming storage time at 4˚C affecting the yield of DNA and thus necessitating the higher freeze storage conditions have been reviewed[4,5], but the genomic content was obtained from microscale volume of blood samples stored at 4˚C, eliminating the necessity of storage conditions at -80˚C.

6. CONCLUSION:

The current study summarizes comparison studies of DNA extraction from stored disease blood samples between standard protocol and optimized protocol**.** The optimized procedure discussed for genomic

DNA extraction from whole blood samples could serve as good reference for such extraction works from stored blood samples.

ABBREVIATIONS:

TKM: Tris HCl-KCl-$MgCl_2$ Buffer
EDTA: Ethylene Diamine Tetra Acetic Acid
TE Buffer: Tris-EDTA buffer
SDS: Sodium Dodecyl Sulphate

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