

Original Research Article

## High-Quality Genomic DNA Extraction from Long Term Stored (LTS) Whole Blood Samples Using Glass Bead Method

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### ABSTRACT

Extracting DNA from long term stored (LTS) whole blood samples remains a challenge, despite numerous attempts to develop a more effective method. Polymerase chain reaction (PCR) success rates with DNA extracted using current methods remain low. In this study, we compared three methods of gDNA extraction methods, from which mechanical (glass bead) method is suitable for gDNA extraction from LTS whole blood sample of coronary artery disease patient. Mechanical (glass bead) method is cost effective with less time consuming method and also gives good yield of gDNA.

**Key words:** LTS whole blood, gDNA, CAD

### INTRODUCTION

DNA is the main raw material to understand the genetics underlying complex disorders in well-characterized patients and the whole blood is the main source for extracting human genomic DNA (gDNA) for experimental use and laboratories purposes. But one of the main disadvantages of DNA extraction from whole blood is that there are not appropriate protocol for extracting gDNA from long term stored (LTS) whole blood.

gDNA extracting through LTS whole blood save the wastage of whole blood sample for further experimental use and laboratories purposes. There are numbers of protocols and kits are available for extracting gDNA from fresh blood but they are not suitable for extracting gDNA from LTS whole blood. Through some protocols, gDNA are extracted from LTS

whole blood but the quality and quantity of DNA are poor. And these methods are also costly and time consuming. Table 1 shows the main categories and subcategories of DNA extraction methods from whole blood samples that are generally used in research facilities worldwide. Laboratory reagents commonly used for each stage of the nucleic acid extraction protocol are included in this table in order to highlight similarities and differences between them.

In the present study, we standardized mechanical (glass beads) method for DNA extraction from LTS whole blood which gives a good quality of DNA with a sufficient quantity of gDNA. This protocol is less time consuming with minimum steps and also economically cheaper than other protocols. So, we compare this protocol with two DNA extracting method viz: salting out (Miller et al, 1988) and kit

method (Geneaid) for blood DNA extraction in 100 LTS whole blood samples collected 6-10 years ago.

## **MATERIALS AND METHODS**

**Samples:** We have randomly selected 50 whole blood samples from CAD patients and 50 healthy controls from Bangalore (Karnataka) and stored it for long-time at -80°C in 5ml EDTA containing vial. All the LTS whole blood samples (50 CAD patient + 50 healthy control) were taken for extracting genomic DNA for study the polymorphism in CAD patient of Bangalore population. This study protocol was approved by Institutional Ethics committee (IEC) of Punjabi University, Patiala.

**gDNA extraction methods:** gDNA were extracted from whole blood samples by three different methods including mechanical (glass bead) method .

**gDNA extraction by salting out method:** gDNA was extracted by standard salting out method (Miller *et al*, 1988). This method involves salting out of the cellular proteins by dehydration and precipitation with a saturated NaCl solution. 1 ml of LTS whole blood were resuspended in 15 ml polypropylene centrifugation tube with 3 ml of nuclei lysis buffer (10 mM Tris-HCl, 400 mM NaCl and 2 mM Na<sub>2</sub>EDTA, pH 8.2). The cell lysates were digested overnight at 37°C with 0.2 ml of 10% SDS and 0.5 ml of a protease K solution (1 mg protease K in 1% SDS and 2 mM Na<sub>2</sub>EDTA). After digestion was complete, 1 ml of saturated NaCl (approximately 6M) was added to each tube and shaken vigorously for 15 seconds, followed by centrifugation at 2500 rpm for 15 minutes. The precipitated protein pellet was left at the bottom of the tube and the supernatant containing the DNA was transferred to another 15 ml polypropylene tube. Exactly 2 volumes of room temperature absolute ethanol were added and the tubes inverted several times until the DNA precipitated. The precipitated DNA strands were removed with a plastic spatula or pipette and transferred to a 1.5 ml micro centrifuge tube containing 100-200 pl TE

buffer (10 mM Tris-HCl, 0.2 mM Na<sub>2</sub>EDTA, pH 7.5). The DNA was allowed to dissolve 2 hours at 37°C before quantitating.

**gDNA extraction by kit:** 300 µl of LTS whole blood taken into a 1.5 ml micro centrifuge tube and add 900 µl of RBC lysis buffer then mix by inverting. Incubate for 5 minutes at room temperature then centrifuge at 3,000 x g for 5 minutes to form a leukocyte (white blood cell) pellet. Remove the supernatant, retaining approximately 50 µl of residual buffer and leukocyte pellet. Vortex the tube until the leukocyte pellet is completely resuspended in the residual buffer. After this add 300 µl of cell lysis buffer to the tube then mix by vortex. Incubate at 60°C for at least 10 minutes to ensure the sample lysate is clear and homogenous. During incubation, invert the tube every 3 minutes. Then add 100 µl of protein removal buffer to the sample lysate then vortex immediately for 10 seconds. Centrifuge at 14,000 x g for 3 minutes to form a tight, dark brown, protein pellet. Now transfer the supernatant to a clean 1.5 ml micro centrifuge tube then add 300 µl of isopropanol and mix well by gently inverting 20 times. Centrifuge at 14,000 x g for 5 minutes then carefully discard the supernatant and add 300 µl of 70% ethanol to wash the pellet. Centrifuge at 14,000 x g for 3 minutes then carefully discard the supernatant and air-dry the pellet for 10 minutes. Finally add 100 µl of DNA hydration buffer then gently vortex for 10 seconds. Incubate at 60°C for 5 minutes to dissolve the DNA pellet.

**gDNA extraction by mechanical (glass bead) method :** 0.5 ml of LTS whole blood were resuspended in 15 ml polypropylene centrifugation tube with 2 ml of lysis buffer (0.3M sucrose, 0.01M Tris-Cl; pH 7.5, 5mM MgCl<sub>2</sub>, 1% triton X100) and spin the centrifugation tube at 35 rpm for 30 minute at room temperature in test tube mixer (rotopin). After this tubes were centrifuged at 3,200 x g for 10 minute at 4<sup>0</sup> C. Now the supernatant was discarded and 1 ml of 10 mM Tris-Cl (pH-8.0) will be added to the

pellet and resuspend the pellet by vigorous vortexing and then go for Centrifugation for 5 minute at 1,000 x g. Repeat this step 2-3 times to remove the RBCs from the pellet. After this supernatant was discarded, 0.5 ml of 10 mM Tris-Cl (pH-8.0); 0.5 ml of laundry powder solution (20 mg/ml); and 20-30 glass beads (0.5mm) were added to each tube. The samples were vortexed for 1 minute, then 0.3 ml of 6M NaCl was added and the samples were vortexed again for 20 seconds. Then the tubes were spun down at 3,200 x g for 20 minutes at 4<sup>0</sup> C. The supernatant was transferred to fresh tubes and DNA was precipitated by the addition of 5 ml of absolute ethanol. Centrifuge the tubes at 3,200 x g for 10 minutes at 4<sup>0</sup> C. Discard the supernatant and wash the pellet with 70% ethanol and finally dissolved in 100 µl of 10mM Tris-Cl (pH-8.0). Incubate at 37°C for 30 minutes to dissolve the DNA pellet.

**Gel Electrophoresis:** The quality of gDNA extracted by each method was assessed by gel electrophoresis. 3 µg of each gDNA extract was analyzed in a 0.8% ethidium bromide agarose gel (Sambrook *et al*, 1989) at 90 V for 30 minute and it was visualized by U.V. Illumination. The intensity of the gDNA bands was quantified using the Gel Doc-It 310 Imaging System.

**Spectrophotometer measurement:** The quantity and purity of gDNA extracted by each method was assessed by spectrophotometric analysis using Eppendorf Biophotometer plus. Each sample was measured at 260/280nm absorbance. The ratio of absorbance at 260nm and 280nm was used to assess protein contamination. gDNA with A<sub>260</sub>/A<sub>280</sub> ratio between 1.8 and 2.0 is considered pure(Nicklas *et al*, 2003).

## RESULTS

**Gel Electrophoresis score:** All the three gDNA extraction methods was compared through Integrity of gDNA band on agarose gel by gel electrophoresis (Fig. 1). All extractions were scored using the Gel Doc-It 310 Imaging System analysis software.

Mechanical (glass bead) method gives satisfactory result, gDNA band was shown in 94 lane of agarose gel out of 100 lane and kit method also gave some result which is very poor with the respect of mechanical (glass bead) method. Kit method gave gDNA band on only 29 lanes out of 100 lanes with poor integrity while salting out gives no result in any sample out of 100 samples.

**Spectrophotometric analysis:** The yield of gDNA extracted through mechanical (glass bead) method was higher than the yield of gDNA extracted through kit method. Mechanical (glass bead) method gave an average of 630 µg/ml while kit method gave only 170 µg/ml. But the purity of gDNA was higher in the kit method. Kit method gave a range of 1.72- 1.94 O.D. Value at 260/280 nm while mechanical (glass bead) method gave a range of 1.47 - 1.91.

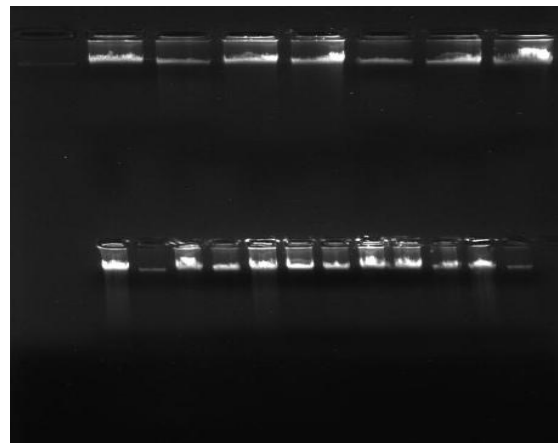


Fig 1 (a): gDNA band of LTS whole blood extracted through mechanical (glass bead) method and run on the 0.8% agarose gel.

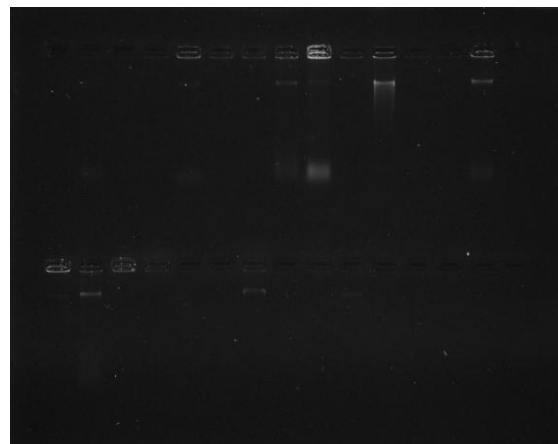


Fig 1 (a): gDNA band of LTS whole blood extracted through kit method and run on the 0.8% agarose gel.

**Table 1: DNA extraction methods commonly used for extraction from whole blood samples**

DNA extraction method (main category)	DNA extraction method (subcategory)	DNA extraction protocol stage			
		Cell lysis	Denaturation of nucleoproteins/ inactivation of cellular enzymes	Removal of contaminants	DNA precipitation
Solution-based DNA extraction methods	Salting out methods	<ul style="list-style-type: none"> <li>• SDS</li> <li>• SDS/proteinase K</li> <li>• Triton X-100</li> </ul>	<ul style="list-style-type: none"> <li>• Proteinase K</li> <li>• Laundry powder</li> </ul>	<ul style="list-style-type: none"> <li>• Potassium acetate</li> <li>• Sodium acetate</li> <li>• Sodium chloride</li> </ul>	<ul style="list-style-type: none"> <li>• Ethanol</li> <li>• Isopropanol</li> </ul>
	Organic solvent/ chaotropes methods	<ul style="list-style-type: none"> <li>• SDS</li> <li>• SDS/proteinase K</li> </ul>	<ul style="list-style-type: none"> <li>• Guanidine thiocyanate</li> <li>• Phenol</li> </ul>	<ul style="list-style-type: none"> <li>• Phenol</li> <li>• Phenol-chloroform</li> <li>• Phenol-chloroform, isoamyl alcohol</li> </ul>	<ul style="list-style-type: none"> <li>• Sodium acetate/ ethanol</li> <li>• Sodium acetate/ isopropanol</li> </ul>
Solid-phase DNA extraction methods	Glass milk/silica resin methods	<ul style="list-style-type: none"> <li>• SDS</li> <li>• Triton X-100</li> </ul>	<ul style="list-style-type: none"> <li>• Guanidine thiocyanate</li> </ul>	<ul style="list-style-type: none"> <li>• Glass milk (silica in chaotropic buffer)</li> <li>• Silica matrix</li> <li>• Diatomaceous earth</li> </ul>	<ul style="list-style-type: none"> <li>• Ethanol</li> <li>• Isopropanol</li> </ul>
	Anion exchange methods	<ul style="list-style-type: none"> <li>• Heat</li> </ul>	<ul style="list-style-type: none"> <li>• Chelex</li> <li>• Chelex/proteinase K</li> </ul>	<ul style="list-style-type: none"> <li>• Chelex</li> </ul>	<ul style="list-style-type: none"> <li>• N/A</li> </ul>
	Magnetic beads methods	<ul style="list-style-type: none"> <li>• SDS</li> </ul>	<ul style="list-style-type: none"> <li>• N/A</li> </ul>	<ul style="list-style-type: none"> <li>• Sodium chloride/ polyethylene glycol</li> </ul>	<ul style="list-style-type: none"> <li>• Magnetic beads</li> </ul>

**Time and cost:** Mechanical (glass bead) method taken approx 2<sup>1</sup>/<sub>2</sub> hour for complete gDNA extraction with number of least step and the cost for extracting gDNA from each sample was approx Rs 20 - 25. On other hand Kit method taken one hour less time than mechanical (glass bead) method but the cost of extracting gDNA was 8 -10 times higher than mechanical (glass bead) method. While salting out take 2 days for extraction and the cost was also higher than mechanical (glass bead) method.

## DISCUSSION

After compare all the three gDNA extraction method from LTS whole blood, mechanical (glass bead) gave the satisfactory result than the other method with good yield of gDNA. The cost of extracting gDNA from LTS whole blood is much cheaper than the other method and quicker method of gDNA extraction from LTS whole blood sample.

**DNA extraction methods using magnetic beads:** Nucleic acid extraction techniques using magnetic separation have been emerging since the early 1990s. They were originally used to extract plasmid DNA from bacterial cell lysates by Hawkins et al in 1994 and in 2006 by Saiyed et al, who

developed and validated a protocol using naked magnetic nanoparticles for genomic DNA extraction from whole blood samples.

Magnetic particles are made of one or several magnetic cores, such as magnetite (Fe<sub>3</sub>O<sub>4</sub>) or maghemite (gamma Fe<sub>2</sub>O<sub>3</sub>), coated with a matrix of polymers, silica, or hydroxyapatite with terminal functionalized groups. In the protocol developed by Saiyed et al, 30 µL of whole blood is mixed with an equal volume of 1% (weight/volume [w/v]) SDS solution. The tube is mixed by inversion two or three times and incubated at room temperature for 1 minute. Ten microliters of magnetic nanoparticles is added to this mixture, followed by the addition of 75 µL of binding buffer (1.25 M sodium chloride and 10% polyethylene glycol 6000). The solution is mixed by inversion and allowed to rest for 3 minutes at room temperature, and the magnetic pellet is immobilized using an external magnet to discard the supernatant. The magnetic pellet is washed with 70% ethanol and dried. The magnetic pellet is resuspended in 50 µL of TE buffer, and magnetic particles bound to DNA are eluted by incubation at 65°C with continuous agitation.



## CONCLUSION

DNA extraction has evolved for the past 145 years and has developed into a diversity of laboratory techniques. There is no consensus on a gold standard method for DNA extraction from whole blood samples, and they all differ in many different aspects. It was concluded that the glass bead method was rapid, economic method for DNA extraction

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