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Cost effective with high quality DNA extraction method from Long Term Stored (LTS) whole blood plasma

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Abstract
Extracting DNA from long term stored (LTS) blood plasma samples remains a challenge, despite numerous attempts to develop a more effective method. In this study, we extracted DNA through glass bead method which is suitable for extraction from LTS whole blood sample of coronary artery disease patient. Glass bead meathod is cost effective with less time consuming method and also gives good yield of DNA.

Keywords: LTS whole blood, DNA, CAD

Introduction
DNA is the main raw material to understand the genetics underlying complex disorders in well-characterized patients and the biological fluid is one of the main sources of DNA extraction for biological and medical experiments. And one of them is blood plasma from where we extracted DNA.
Blood plasma is the yellowish coloured liquid portion of the whole blood after removing the red blood cells, white blood cells, platelets and other cellular components from the blood. But one of the main disadvantage of DNA extraction from blood plasma is that there are not appropriate protocol for extracting DNA from long term stored (LTS) blood plasma.
DNA extracting through LTS blood plasma 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 DNA from fresh blood plasma but they are not suitable for extracting DNA from LTS blood plasma. Through some protocols, DNA are extracted from LTS blood plasma but the quality and quantity of DNA are poor. And these methods are also costly and time consuming.
In the present study, we standarized glass beads method (Jha *et al*, 2016) for DNA extraction from LTS blood plasma which gives a good quality of DNA with a sufficient quantity of DNA. This protocol is less time consuming with minimum steps and also economically cheaper than other protocols.

Materials and method
Samples
We have randomly selected 50 whole blood samples from CAD patients from bangalore (Karnataka) and separate plasma and stored it for long-time at -80^{o} C in 1.5ml tube.All the LTS blood plasma samples (50 CAD patient) were taken for extracting 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.

Separation of Plasma from whole blood
Centrifuge the tube containing whole blood at 2000 rpm for 10 minutes at 4^{0}C. Then remove 1 ml of plasma from the tube into fresh 1.5 ml tube and stored it at -80^{o} C

DNA extraction from plasma by glass bead method
DNA was extracted by glass bead method. 1 ml of LTS blood plasma 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 $MgCl_2$, 1% triton X100) and spin the centrifugation tube at 35 rpm for 30 minute at room temperature in test tube mixer (rotospin). After this tubes were centrifuged at 3,200 x g for 10 minute at 4^0 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 vigrous vortexing and then go for Centrifugation for 5 minute at 1,000 x g. 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 4^0 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 4^0 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 37ºC for 30 minutes to dissolve the DNA pellet.

Gel Electrophoresis

The quality of DNA was assessed by gel electrophoresis. 3 µg of each DNA 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 DNA bands was quantified using the Gel Doc-It 310 Imaging System.

Spectrophotometer measurement

The quantity and purity of DNA was assessed by spectro-photometeric analysis using Eppendorf Biophotometer plus. Each sample was mesured at 260/280 nm absorbance (Sambrook *et al*, 1989). The ratio of absorbance at 260 nm and 280 nm was used to assess protein contamination. DNA with A260/280 nm ratio between 1.8 and 2.0 is considered pure (Nicklas et al, 2003).

Results

Gel Electrophoresis score

All the DNA extraction samples was measured through Integrity of DNA band on agarose gel by gel electrophoresis (Fig. 1) and extractions were scored using the Gel Doc-It 310 Imaging System analysis software. Glass bead method gives good result, DNA band was shown in 92% of lanes.

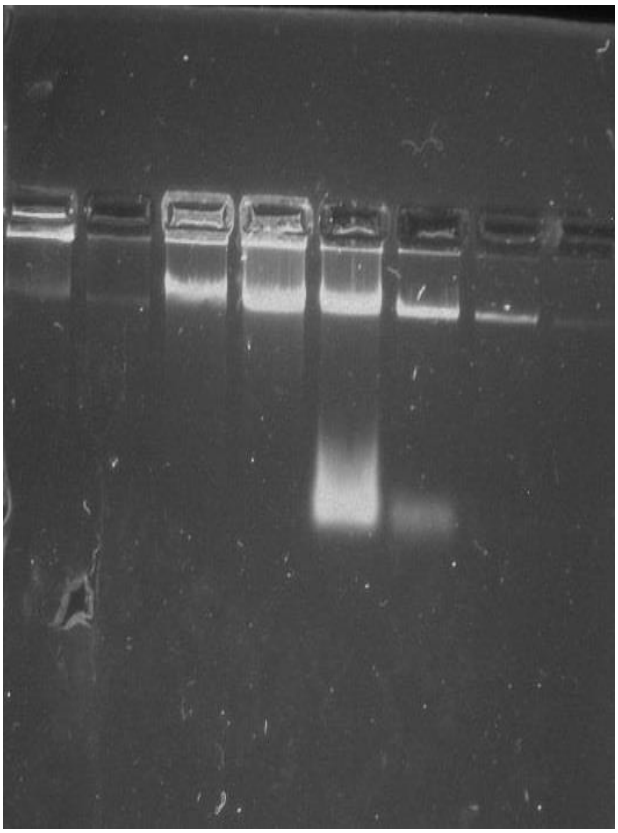

Fig. 1: DNA band of LTS blood plasma extracted through glass bead method and run on the 0.8% agrose gel.

Spectrophotometeric analysis

The yield of DNA extracted through Glass bead method gave a average of 570 µg/ml and gave a range of 1.59-1.83 O.D. Value at 260/280 nm.

Time and cost

Glass bead method taken approx $2^1/_2$ hour for complete DNA extraction with number of least step and the cost for extracting DNA from each sample was approx Rs 20 - 25.

Discussion

DNA extraction from LTS whole blood plasma, glass bead method gave the satisfactory result with good yield of DNA. The cost of extracting DNA from LTS whole blood plasma is much cheaper than the other method and this method is also quiker method of DNA extraction from LTS whole blood plasma sample.

References

1. Jha, C.K., Chahal, S.M.S., Khullar, N et al. High-quality genomic DNA extraction from long term

stored (LTS) whole blood samples using glass bead method. Int J Health Sci Res. 2016; 6(5):288-292.
2. Nicklas, J.A., Buel, E. Quantification of DNA in forensic samples. Anal Bioanal Chem 2003;376:1160e7.
3. Sambrook, J.E.F., Fritsch. E.F. and Maniatis, T. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York.