

original article

Comparative study of five methods for DNA extraction from whole blood samples

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ABSTRACT**Objectives**

To find a suitable DNA isolation system from whole blood; five different DNA extraction methods were evaluated, comparing manual, partially automated and fully automated separation.

Methods

Phenol-chloroform, simple extraction by microwaving and the Wizard Genomic DNA Purification system were compared in this study. In addition to these manual methods, the MagNA Pure platform was performed manually, partially automated and fully automated. The last technique was a combination between the Wizard SV 96 Genomic DNA Purification Kit and the MagNA Pure extraction in which the whole vacuum system for waste collection was replaced by the magnetic separation principals.

Results

Best results were observed with the MagNA Pure automatic extraction that obtained nucleic acid amount of 0.98 µg/µl with 2.91 purity ratio. The second best methods were the magnetic beads based techniques, either the original or the modified one which showed purity ratios, 2.45 and 2.36 respectively and the amount of the nucleic acids recovered were 0.79 µg/µl and 0.76 µg/µl respectively.

Conclusions

Magnetic DNA separation is recommended for its high-quality nucleic acid extraction in which the risk of cross-contamination is reduced. It requires minimal starting material and is both cost-effective, user friendly and can be optionally automated with no manual intervention steps.

Keywords: DNA, isolation and purification, whole blood

INTRODUCTION

Genomic DNA extraction is essential step prior to wide range molecular applications regardless of the molecular system chosen. DNA extraction method not only should yield DNA

suitable for DNA quantity specifications but should provide the required amount of high-quality DNA with minimal contaminants which may interfere with the molecular technique.¹ The basic steps of DNA isolation are disruption of the cellular structure to create a lysate, separation of the soluble DNA from cell debris and other insoluble material and purification of this DNA from soluble proteins and other nucleic acids. Disruption of most cells is done by chaotropic salts, detergents or alkaline denaturation, and the resulting lysate is usually cleared by centrifugation, filtration or magnetic clearing. Historically, extraction of DNA was done using organic extraction (e.g. *phenol-chloroform extraction, a liquid-liquid technique*) followed by ethanol precipitation.² Other low cost laboratory methods were also used as thermal shock by boiling or microwaving.³ On the other hand, a variety of commercially available kits are widely applied either manually, partially automated or by using a fully automated system.

In a trial to find a suitable DNA isolation system from whole blood, five different DNA extraction methods were evaluated in the present study ranging between manual, partially automated and fully automated separation.

MATERIALS AND METHODS

96 blood samples, 2 ml each, were collected within one week on EDTA tubes from patients attending the outpatient clinic in Taibah University, Saudi Arabia, for routine checking.

The DNA extraction methods used were as follows;

- phenol-chloroform purification method followed by an ethanol precipitation²,
- simple extraction by microwaving based on thermal shock³ and
- DNA extraction with the Wizard Genomic DNA Purification Kit (Promega Corporation, Madison, WI, USA), which was carried out following the manufacturer's instructions.
- In addition to these three manual methods, the fully automated MagNA Pure platform based on magnetic glass (silica) particles was performed using LC Magna Pure Compact Instrument (Roche Diagnostics, Hoffmann-La Roche Ltd, USA).
- The previous technique was also applied manually and partially automated using the Precision™ XS Microplate Sample Processor from *BioTek Instruments* (Inc., P.O. Box 998, Highland Park, Winooski, Vermont 05404-0998 USA) for quick automatic washing.

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