e-ISSN - 2348-2184
Print ISSN - 2348-2176

AMERICAN JOURNAL OF BIOLOGICAL AND PHARMACEUTICAL RESEARCH

Journal homepage: www.mcmed.us/journal/ajbpr

EVALUATION OF FOUR DNA EXTRACTION METHODS FROM WHOLE BLOOD SAMPLES

Samar Osman, Huzifa Awad A kareem, Elamin Abdelkreem*

Department of Medical Parasitology, Faculty of Medical Laboratory Sciences, Al Neelain University, Khartoum, Sudan.

Article Info
Received 29/04/2015
Revised 16/05/2015
Accepted 09/06/2015

Key words: -
DNA extraction methods, phenol-chloroform-isoamyl, guanidine hydrochloride, saturated sodium chloride, and chelex-100

ABSTRACT
In most studies about molecular genetics, molecular diagnostics, DNA extraction is considered as the very first step that will give a significant effective. The material used for DNA extraction is whole blood, it aimed to evaluate and compare between four DNA extraction methods, including phenol-chloroform-isoamyl, guanidine hydrochloride, saturated sodium chloride, and chelex-100 using 50 blood samples collected from healthy faculty students, The comparison included DNA concentration, extracted DNA purity, cost reagents per one sample, and time consuming. The result showed that, the highest mean of DNA concentration was obtained by using saturated NaCl (76.164 µg/ml), by chelex-100 (45.322µg/ml), phenol-chloroform-isoamayle (44.68µg/ml) and lastly guanidine hydrochloride (14.438µg/ml). The mean of optical densities 260nm/280nm ratio was calculated to assess extracted DNA purity, it was maximum in case of Chelex-100 (1.288), phenol-chloroform-isoamayle (1.786), saturated NaCl (1.884), and lastly guanidine hydrochloride (2.002), In term of cost per one sample, guanidine hydrochloride was the highly cost method (15 SDG) followed by chelex-100 (10 SDG), then phenol-chloroform-isoamyle (6 SDG) and lastly saturated NaCl (4 SDG).Guanidine hydrochloride took the longest time for DNA extraction (4 days), phenol-chloroform-isoamayle (3 days), saturated NaCl (2 day), and chelex-100 took one day (two hours). After a comprehensive analysis of all factors: salt extraction gave the maximum yield of DNA, it is saver and simpler than the other methods. Moreover, this method is reliable and inexpensive. But its purity is slightly low.

INTRODUCTION

Over the years several well established techniques have been developed with varying quantities and quality of DNA extracted. However, such procedures often involve tricky or tedious steps and cannot be used on large scale basis [1]. In the DNA extraction process, the cells must be broken to obtain DNA. The particles present inside the cell are disrupted and their alignment is broken.

Corresponding Author

Elamin Abdelkarim Elamin
Email:- elaminpara72@yahoo.com

There are several methods for the release of nucleic acid from microorganisms, such as boiling in distilled water or PCR buffer [2], detergents with or without heat [3], sodium hydroxide with heat [4], freeze-thaw [4], SDS-proteinase K [5], percloric acid [6], enzymes [7], sonications, and heat [8]. After cell lysis, the lipids present in cells are removed by the means of a detergent. The next step is to remove the proteins using protease. Finally, the DNA is precipitated using an alcohol. Alcohols that are commonly used in precipitation process are isopropanol or ice cold ethanol. To precipitate DNA, centrifugation of the solution containing DNA is carried out. It not only separates DNA, but also removes salts from the solution [9].

Several DNA extraction methods are widely used to extract DNA including phenol extraction. Although precipitation with phenol-chloroform provides DNA fragments of high molecular weight sufficient for any application of molecular biology, this method involves many slow and costly steps. It also includes the use of potentially health hazardous chemicals (phenol and chloroform) which make this process difficult to carry out on a large scale in laboratory routine work [8].

An alternative to this method, solid phase carriers (Silica matrix, glass particles), ion exchange resins, or magnetic particles and column application strategies have been developed as new protocols for of DNA extraction [10].

It is not only essential to obtain a high yield and quality of DNA, but also to the effectively removal of contaminants that can affect PCR reactions and these may even be present in the components of DNA extraction solutions [9].

The extraction and isolation of DNA from blood is a necessary step for PCR-based analysis and a key factor in determining the overall efficiency and reliability of any PCR-based test [11].

These difficulties have resulted in the popular usage of commercial kits with standard and guided protocols [9]. Recently, many commercial kits for nucleic acid extraction from clinical specimens have been introduced [12]. All extraction methods from commercial kits are easier to perform, safer and more rapid, although there are slight differences in the hands on time from one to another [13].

Although, these commercial kits have provided a certain degree of reliability, most laboratories are either unaware of the alternatives or are hesitant to utilize them. The reasons for this may be the lack of literature that assesses the cost effectiveness of different techniques and the general perception regarding imported goods from developed countries [14, 15].

This study aimed to evaluate four DNA extraction methods including phenol chloroform isoamyl, guanidine hydrochloride, salting out method and Chelex® in term of concentration, purity, time and cost

MATERIALS AND METHODS

A total of 50 blood samples were used in the study from each individual, ten ml of blood sample were collected into blood container containing EDTA, after application of standard non traumatic vein puncture. The sample was stored at 4 ºC until been processed.

Each blood sample was mixed with double volume of red blood cells lysing buffer in 50 ml falcon tube, centrifuged for 10 minutes at 3000 rpm to pellet the white blood cells. The supernatant was discarded and the cell pellet was centrifuged again with red blood cells lysing buffer till the white blood cells precipitated as white pellet. For each sample, 8 µl proteinase K and 2 ml of nuclear lysis buffer were added, vortexed and mixed thoroughly and incubated over night at 37ºC.

The digest of white blood cells was then dispensed equally into four 15 ml falcon tubes and stored at 4 ºC ready for protein precipitation. Cellular proteins were precipitated using four DNA extraction protocols: Phenol-chloroform-isoamyl alcohol, saturated sodium chloride, and guanidine hydrochloride and chelex-100 extraction.

Extraction methods

Phenol - chloroform – isoamyl alcohol

Equal volume of phenol-chloroform-isoamyl alcohol prepared as 25:24:1 was added to the sample lysate, mixed and centrifuged at 6000 rpm for 5 minutes.

The upper layer was transferred in to a clean tube, and equal volume of chloroform -isoamyl alcohol was added, mixed and centrifuged at 6000 rpm for 5 minutes.

The upper layer was transferred to clean tube and DNA was precipitated by adding two volumes of cold ethanol and 1:10 of sample volume of 3 M sodium acetate and incubated at -20ºC overnight.

The sample was centrifuged for 10 minute at 12000 rpm and the supernatant was discarded. Two ml of 70% ethanol was added, mixed well and centrifuged for 7 minutes at 12000 rpm, and then supernatant was discarded. This step was repeated. The pellet was allowed to dry overnight. DNA was suspend in 200 µl of distilled water (dH_2O), incubated in 40 ºC oven for couple of hours to dissolve the DNA and stored at -20ºC.

Guanidine hydrochloride

Sample lysate was warmed to room temperature and 1 ml of 5 M guanidine hydrochloride and 300 µl of ammonium acetate were added and mixed well, incubated at 37 ºC overnight. 2 ml of pre chilled chloroform was added, vortexed and then centrifuged for 5 minutes at 2500 rpm. The upper layer was collected in to a new tube and double volume of cold absolute ethanol was added, mixed and incubated at -20ºC overnight. The sample was then centrifuged at 6000 rpm for 20 minutes, and the supernatant was carefully drained; the pellet was washed with 70% ethanol and centrifuged at 6000 rpm for 15 minutes. The supernatant was discarded and the pellet was allowed to dry overnight. The pellet was resuspended in 200µl dH_2O, vortexed and placed in 40ºC oven for a couple of hours to dissolve the DNA and stored at -20 ºC.

Saturated sodium chloride (NaCl)

Sample lysate was warmed to room temperature and 1 ml of 5 M NaCl was added, vortexed and then centrifuged for 10 minutes at 2500 rpm. Supernatant was trans fared to another container and to that double volume of cold ethanol was added to precipitate DNA. Centrifuged at 6000 rpm for 20 minutes, and the supernatant was drained; the pellet was washed with 70% ethanol and

centrifuged at 6000 rpm for 15 minutes. The supernatant was discarded and the pellet was allowed to dry overnight. The pellet was suspended in 200µl dH_2O, vortexed and placed in 40ºC oven for a couple of hours to dissolve the DNA and then stored at -20 ºC. [16]

Chelex -100 resin extraction

1 ml of hot 10% Chelex-100 resin was added to sample lysate, vortexed for15-20 seconds and the mixture was incubated at 100ºC for 20 minutes. The tube was vortexed again for 10-15 seconds and then centrifuged at high speed in microcentrifuge at 10,000 rpm for 10 minutes. The supernatant containing the DNA was stored at -20 ºC [10]. The concentration and purity of DNA were estimated using Nanodrope®. DNA purity was obtained by calculating optical density ratio at 260 nm/280 nm. Smaller ratios indicate contamination with proteins, phenol or others interfering compounds which strongly absorbed at 280 nm [17].

Ethical clearance

The study was approved by the board of the ethics of Faculty of Medical Laboratory Science**s.**

Data analysis

Results obtained were analysed by computerized Program of Statistical Package for Social Studies (SPSS) version 13.0,

RESULTS

Result of DNA concentration and purity

We tabulate the results and calculate the mean of concentrations and standard deviation. Saturated NaCl give the highest mean of concentration followed by Chelex-100 extraction, then phenol-chloroform-isoamyl and guanidine hydrochloride which give the lowest mean of concentration.

The mean of 260nm/280nm ratio was calculated to assess the purity of the DNA; it gave maximum readings by chelex-100, followed by phenol-chloroform-isoamyl, saturated NaCl, and guanidine hydrochloride respectively (Table 1)

Statistical analysis of data

Statistical analysis of data using paired samples test show that there are significant differences in the DNA concentration obtained by all protocols except between chelex-100 and phenol-chloroform (Table 2).

In term of purity, there are no significant differences between all protocols except guanidine hydrochloride and all other protocols (Table 3).

In term of cost, guanidine hydrochloride is the most expensive method followed by chelex-100, phenol-chloroform and lastly saturated NaCl. Guanidine hydrochloride is highly time consuming Followed by phenol-chloroform-isoamyl, saturated NaCl and chelex-100 (Table 4).

Table 1. Mean of DNA concentration and purity

	DNA Concentration (µg/ml)		DNA Purity (260nm/280nm)	
Extraction Method	**Mean**	**Standard Deviation**	**Mean**	**Standard Deviation**
Chelex-100	45.322	16.3733	1.288	0.1081
	76.164	66.8153	1.884	0.1346
	44.68	24.2449	1.786	0.1702
	14.438	18.2452	2.002	0.9065

Table 2. Paired Samples Test for concentration

Paired Differences				Sig
Mean		**Std. Deviation**	**Std. Error Mean**	
NaCl - Phenol	31.4840	59.1019	8.3583	.000
NaCl - Guanidine	61.7260	61.7492	8.7326	.000
NaCl – Chelex-100	30.8420	62.8176	8.8837	.001
Phenol -Guanidine	30.2420	28.1741	3.9844	.000
Phenol-Chelex100	-.6420	19.1038	2.7017	.813
Guanidine – Chelex100	-30.8840	23.1827	3.2785	.000

Table 3. Paired Samples Test for DNA purity: Result of cost and time consuming

Paired Differences				Sig
Mean		**Std. Deviation**	**Std. Error Mean**	
NaCl - Phenol	.0980	.2190	.0310	.003
NaCl - Guanidine	-.1180	18.2512	2.5811	.368
NaCl - Chelex-100	.5960	.1807	.02255	.000
Phenol - Guanidine	-.2160	.9182	.1298	.103
Phenol- Chelex-100	.4980	.1672	.0236	.000
Guanidine – Chelex100	.7140	.9058	.1281	.644

Table 4. Cost and time consuming of DNA extraction methods

Extraction method	Cost/one sample	Time
Chelex-100	10 SDG	2 hours
Saturated NaCl	4 SDG	2 day
Phenol-chloroform	6 SDG	3 days
Guanidine hydrochloride	15 SDG	4 days

DISCUSSION AND CONCLUSION

The choice of DNA extraction method requires an evaluation of several factors. The most suitable extraction procedure should be financially and practically applicable and deliver accurate results.

The statistical analysis of this study showed that the DNA quantity obtained was significantly different between all protocols except the oldest one, phenol-chloroform-isoamyle and chelex-100. With saturated NaCl extraction gave the highest quantity of DNA extracted from blood samples, followed by Chelex-100 extraction and phenol-chloroform-isoamyle and lastly guanidine hydrochloride.

REFERENCES

1. Adhya S, Chatterjee M, Hassan MQ, Mukherjee S, Sen S. (1995). Detection of Leishmania in the blood of early kala-azar patients with the aid of the polymerase chain reaction. *Trans R Soc Trop Med Hyg*, 89(6), 622-4.
2. Mackay IM, Arden KE, Nitsche A. (1989). Real-time PCR in virology. Nucleic Acids Res. 2002 Mar 15; 30(6):1292-305.
3. Kwok S, Higuchi R. Avoiding false positives with PCR. *Nature*, 339(6221), 237-8.
4. Furrer B, Candrian U, Wieland P, Lüthy J. (1990). Improving PCR efficiency. *Nature*, 346(6282), 324.
5. Muralidhar B, Steinman CR. (1992). Geometric differences allow differential enzymatic inactivation of PCR product and genomic targets. *Gene*, 117(1), 107-12.
6. Longo MC, Berninger MS, Hartley JL. (1990). Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. *Gene*, 93(1), 125-8.
7. Lo YM, Wainscoat JS, Fleming KA. (1994). Non-invasive prenatal diagnosis. Lancet, Mar 26, 343(8900), 802-3.
8. Anthony JR, Green HA, Donohue TJ. (2003). Purification of Rhodobacter sphaeroides RNA polymerase and its sigma factors. Methods Enzymol, 370, 54-65.
9. Mahittikorn A, Wickert H, Sukthana Y. (2005). Comparison of five DNA extraction methods and optimization of a b1 gene nested PCR (nPCR) for detection of Toxoplasma gondii tissue cyst in mouse brain. *Southeast Asian J Trop Med Public Health*, 36(6), 1377-82.
10. Garcia Gonzalez LA, Rodrigo Tapia JP, Sánchez Lazo P, Ramos S, Suárez Nieto C *et al*. (2004). DNA extraction using Chelex resin for oncogenic amplification analysis in head and neck tumours. *Acta Otorrinolaringol Esp*, 55(3), 139-44.
11. Kubista M, Andrade JM, Bengtsson M, Forootan A, Jonák J, Lind K. (2006). The real-time polymerase chain reaction. *Mol Aspects Med*, 27(2-3), 95-125.
12. Wilson IG. (1997). Inhibition and facilitation of nucleic acid amplification. *Appl Environ Microbiol*, 63(10), 3741-51.
13. Riemann K, Adamzik M, Frauenrath S, Egensperger R, Schmid KW, Brockmeyer NH, Siffert W. (2007). Comparison of manual and automated nucleic acid extraction from whole-blood samples. *J Clin Lab Anal*, 21(4), 244-8.
14. Syed M, Zaidi A, Moin O, Bhatti F. (2008). Comparison of methods for DNA extraction in a low cost laboratory setting for developing countries. *Pak J Med Sci*, 19, 6-11.
15. Joshi CG**,** Rank DN, Brahmkshtrl BP, Patel AV, Vataliya PH. (1998). Comprison of DNA extraction protocols. *Indian Veterinary Journal*, 75, 1037-2.
16. Miller S A *et al. (*1988). A Simple Salting out Procedure for Extracting DNA from Human Nucleated Cells". *Nucleic Acids Res*, 16, 1215.
17. Sameer AS**,** Rehman SU, Banday MZ, Syeed N, Pandit AA, Nanda MS *et al*. (2009). Comparison of the different methods of DNA extraction from snap-frozen tumor tissues. *IJBB*, 18, 218, 902.