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Comparison of the quality of the DNA extracted from different media at the Laboratory of molecular biology of the faculty of medicine of UNIKIN

Abstract

Background: Since the advent of new techniques, Molecular Biology has significantly improved routine diagnostic methods in medicine. Its medical applications are often carried out thanks to the study of the Deoxyribose Nucleic Acid (DNA).

Objective: The objective of this study is to compare the quality of DNA extracted from whole blood in a tube and that extracted from Dried Blotting Paper (DBS).

Methodology: Experimental study carried out in the Laboratory of Molecular Biology on 50 samples of 5 ml of blood taken from tubes with EDTA anticoagulant. The samples were aliquoted into 2 tubes A and B of equal volume. The blood samples in tube B were centrifuged to obtain a 3-phase separation and prepare buffy coat. Two hundred microliters were taken from each tube and placed on blotting paper. The protocol used to extract DNA is the QIAamp® DNA Blood Mini kit. The concentration of the DNA is measured with an absorbance at 260nm and the quality evaluated by the A260/A280 ratio.

Results: The Buffy Coat gave a better DNA concentration than whole blood regardless of the extraction medium. The quality of the extracted DNA is comparable in the different media; nevertheless, the liquid Buffy Coat gave a slightly better quality compared to the other extraction media.

Conclusion: The DNA extracted in the Molecular Biology laboratory of the Faculty of Medicine of UNIKIN is pure and of good quality. The Buffy coat produces more DNA than whole blood. Blotting paper is a good alternative because it gives comparable results with liquid material.

Keywords: DNA, extraction, whole blood, blotting paper, molecular biology

Volume 4 Issue 1 - 2019

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Received: December 19, 2018 | **Published:** February 28, 2019

Introduction

Since the advent of the Polymerization Chain Reaction (PCR) technique, Molecular Biology has considerably improved routine diagnostic methods in medicine in general. Its medical applications (molecular and antenatal diagnosis of infectious, hereditary diseases, genotyping, etc.) are often carried out through the study of Deoxyribose Nucleic Acid (DNA), which is the fundamental genetic material that governs the principles of Molecular Biology. Nevertheless, the study of Ribose Nucleic Acid (RNA) is also necessary in certain cases such as quantification of viral RNA (Viral Load).

DNA is usually extracted from the blood leukocytes, but can also be obtained from certain biopsies or cell cultures. In most cases, DNA extraction techniques must be adapted to the type of biological sample, the nature of the genome to be extracted, the number of copies in the sample and the techniques of molecular biology to be used.[1]

In recent years, several nucleic acid extraction procedures (DNA and RNA) have been explored and various commercial extraction kits have been developed by different firms. However, the primary objective remains to extract, purify and eliminate the contaminants for the various reactions to be carried out thereafter from the sample.

The aim of this study is to compare the quality of DNA extracted from blood in a tube and that extracted from Dried Blotting Paper (DBS).

Methodology

The present work was an experimental study carried out in the laboratory of Molecular Biology of the Faculty of Medicine of the University of Kinshasa (UNIKIN). Fifty blood samples were collected in the tubes with anticoagulant EDTA. The samples had been drawn from volunteers after the informed consent had been obtained.

Five milliliters of blood were collected in a tube with EDTA from the vein in the fold of the elbow. Each blood sample was aliquoted into 2 tubes of 2.5ml (Tube A and B). The tube-B was used for the preparation of the Buffy Coat; then 200µl of the buffy coat was deposited on the DBS. The whole blood in the tube-A was homogenized and then 200µl of the solution was deposited on the DBS.

Preparation of buffy coat

The blood samples in tube-B were centrifuged at 3000 rpm for 10 minutes to obtain a three-phase separation: plasma, cell pellet and Buffy coat (the white ring between the supernatant and the pellet). Five hundred microlitre (500µl) of the buffy coat was then transferred into a previously labeled tube.

Marking on blotting paper (DBS)

Two hundred microliter (200µl) of whole blood (Tube-A) and Buffy coat (Tube-B) were taken from the freshly collected tubes

and deposited on previously labeled blotting paper. The volume was laid on the blotting paper by drawing circles and without piercing or crumbling the support.

Extraction of DNA

The protocol used to extract the DNA is that of the kit QIAamp DNA Mini and Blood Mini proposed by the manufacturer.[2] For this work, the incubation times during the extractions were extended in accordance with the recommendations of the manufacturer. The extraction was carried out at the Laboratory of Molecular Biology of the Department of Basic Sciences of the Faculty of Medicine of UNIKIN from 200µl of whole blood or Buffy coat and a circle 1cm in diameter on blotting paper. An eluate of 200µl was collected from each extraction.

Spectrophotometry of DNA

The concentration of the double-stranded DNA was measured from an Ultra-Violet spectrophotometer (Spectronic Genesys Bio, Thermo Electron Corporation) by an absorbance at 260nm. The quality of the extracted DNA is evaluated by the ratio A260/A280. This report provides a good indication of the purity of the extracted sample. Indeed, the presence of proteins in a sample increases the value at A280. A value between 1.8 and 2 indicates that the extracted DNA is pure whereas a lower value indicates the presence of protein contamination.[3]

Results

For the 50 blood samples obtained, 200 eluate of 200µl each were collected according to 4 eluates (1 whole blood liquid, 1 whole blood on blotting paper, 1 liquid Buffy coat and 1 Buffy coat on blotting paper) for each sample. Buffy Coat gave a better DNA concentration than whole blood regardless of extraction medium (Table 1). The quality of the extracted DNA is comparable in the different media. The liquid Buffy Coat gave a slightly higher quality compared to the other extraction media (Table 1).

Table 1 Comparison of concentration of extracted DNA

	Liquid material		Blotting paper (DBS)	
	Whole blood	**Buffy coat**	**Whole blood**	**Buffy coat**
Concentration average (ng/µl)	49.8	245.8	42.8	239.6
A260/A280	1.89	1.93	1.92	1.95

Discussion

The aim of this study was to compare the quality of DNA extracted from blood in a tube and that extracted from dried blotting paper (DBS) at the Laboratory of Molecular Biology of the Faculty of Medicine of the University of Kinshasa (UNIKIN). Fifty (50) blood samples were obtained from a routine laboratory testing center for this work. Two hundred (200) eluate of 200µl each were extracted in order of 4 eluates (1 whole blood, 1 whole blood on blotting paper, 1 liquid Buffy coat and 1 buffy coat on blotting paper) for each sample.

After DNA extraction and spectrophotometric reading, liquid Buffy Coat samples gave average DNA concentrations of 245.8ng/µl and 239.6ng/µl from liquid and blotting paper (DBS) respectively. Whole blood gave concentrations of 49.8ng/µl and 42.8ng/µl respectively from liquid material and DBS. This is explained because the Buffy coat is the enriched fraction of Leukocytes in a collection of whole blood. Hence, DNA extraction on Buffy coat should logically yield 5 to 10 times more DNA than its equivalent in blood volume.[2,4] The quality of the extracted DNA is higher than the acceptable average; the mean concentration of DNA extracted on 200µl volume, according to the manufacturer QIAGEN, is 34.0ng/µl with the kit QIAGEN® DNA Blood Mini.[2] This difference in concentrations is due to prolonged incubation times and extraction media.

The DNA extracted is pure and of good quality. DBS gave DNA grades of 1.92 and 1.95 respectively for whole blood and Buffy coat. The liquid material gave DNA grades of 1.89 and 1.93 respectively for whole blood and Buffy coat. The quality of the extracted DNA is comparable in the different media; nevertheless, the Buffy Coat DBS gave a higher quality compared to the other extraction media. The A260/A280 ratio indicates the purity and quality of the DNA samples. The closer it gets to 2, the less protein contamination the sample has. A pure and good quality DNA should have an A260/A280 ratio of between 1.8 and 2.

Although the extraction of genetic material from blotting paper is laborious and long enough, the quality of the extracted material is slightly superior to liquid blood.[4]

Conclusion

The Deoxyribose Nucleic Acid (DNA) extracted in the Molecular Biology Laboratory of the Faculty of Medicine is pure and of good quality. The Buffy coat produces more DNA than whole blood. Blotting paper is a good alternative to liquid material because it gives comparable results with it.

Acknowledgments

None.

Conflicts of interest

The author declares there is no conflicts of interest.

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