

A COMPARATIVE STUDY OF TWO METHODS FOR THE ISOLATION OF HUMAN LEUCOCYTES FOR DNA EXTRACTION

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Summary

The 'Dextran' and the 'Buffy-coat' methods for isolation of human leucocytes for DNA extraction were compared on the basis of DNA yield from the same amounts (10 ml) of blood. Human leucocytes from a total of 11 samples were isolated using both methods for each sample after which DNA was extracted. Extracted DNA samples were treated with ribonucleases and proteinase K after which the yields were quantitated by measuring absorbance at 260 nm. The 'Buffy-coat' method yielded a mean concentration of DNA of 476.7 $\mu\text{g/ml}$ (range: 212 to 700 $\mu\text{g/ml}$) while the 'Dextran' method yielded 188.4 $\mu\text{g/ml}$ (range: 64 to 340 $\mu\text{g/ml}$). The difference was confirmed by subjecting the extracted DNA samples to agarose gel electrophoresis.

INTRODUCTION

DNA analysis for genetic and infectious diseases is emerging as a modern diagnostic tool. As such, there is a need for a simple and efficient method for the isolation of sufficient genomic DNA which can then be manipulated using restriction endonucleases.

There are various methods for the isolation of DNA from biological specimens. The 'Dextran' and 'Buffy-coat' methods, employed in this comparative study involved the isolation of human leucocytes for DNA extraction using phenol. These methods were modified from the standard DNA isolation procedure.²

MATERIALS AND METHODS

11 samples, 9 from normal individuals and 2 from individuals with beta-thalassemic trait, were used in this comparative study. 20 ml of heparinised peripheral blood was obtained from each individual and, from it, 10 ml was used for each extraction method.

In the 'Dextran' method (Figs. 1 & 2) 1 ml of dextran was added to the heparinised blood and incubated at 30°C for 1 hr. After incubation, the upper layer containing the leucocytes was separated from the RBC layer and spun at 2283 g for 20 mins at 4°C to obtain the leucocyte button. The leucocyte button was washed in Tris-EDTA (TE) buffer to remove contaminant RBC. It was then digested overnight at 37°C with proteinase K in TE buffer. After incubation, DNA was extracted by phenol and spun at 6800 g for 10 mins at room temperature. The DNA in the upper layer was then precipitated in absolute alcohol at -20°C overnight and spun at 8000 g for 15 mins. The DNA precipitated was

freeze-dried, dissolved in distilled H₂O and stored at -20°C.

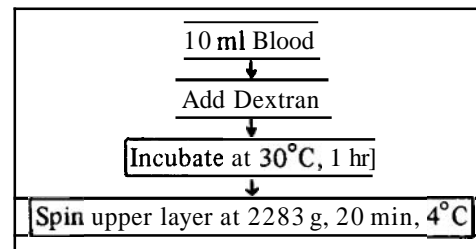


FIG. 1: Initial procedure of 'Dextran' method for isolation of blood leucocytes.

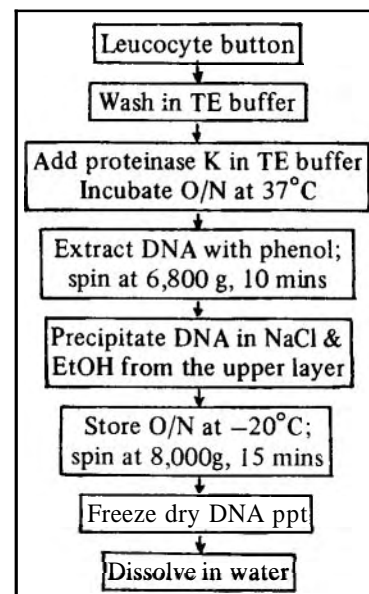


FIG. 2: DNA extraction procedure

The 'Buffy-coat' method (Figs. 2 & 3) differs from the 'Dextran' method in the initial steps of obtaining the leucocyte button. Heparinised blood was washed 3 times in 0.15M NaCl after which the buffy-coat was carefully pipetted out. Contaminating RBC was then lysed in proportionate volumes of 0.144M NH_4Cl and 0.01M NH_4HCO_3 . The leucocyte button was obtained by spinning at 2283 g for 20 mins at 4°C and washed in TE buffer. The subsequent DNA extraction was the same as in the 'Dextran' method.

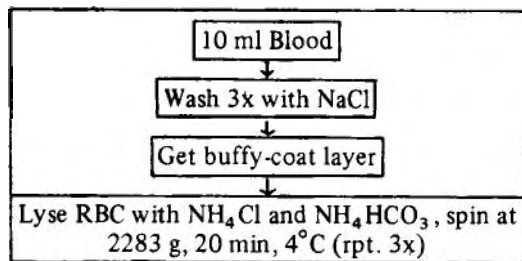


FIG. 3: Initial procedure of 'Buffy-coat' method for isolation of blood leukocytes.

Digestion with RNase

In both methods, RNA was extracted together with DNA. To remove the RNA, the samples were digested with ribonuclease T1 and A.

Quantitation of DNA

Each RNase digested DNA specimen was quantitated by measuring its absorbance at 260 nm. The amount of DNA (ug/ml) present was calculated on the assumption that 50 ug/ml of DNA gave an optical density at 260 nm of 1.

Visualization of DNA separation

To visualize DNA separation, 1 ul of each RNase-treated DNA sample was subjected to 1% agarose gel electrophoresis after which the gel was stained in ethidium bromide for visualization under UV. Ethidium bromide is an intercalating dye and as little as 0.05 ug DNA can be detected by direct examination of the gel in UV light as it gives out an orange fluorescence in the presence of DNA.

RESULTS

The DNA yields after RNase digestion using the two methods are shown in Table 1.

TABLE 1
DNA YIELDS FROM 'DEXTRAN' AND 'BUFFY-COAT' METHODS

Specimen	DNA yield (ug/ml)	
	'Dextran' method	'Buffy-coat' method
1	172	500
2	136	412
3	340	668
4	64	212
5	164	320
6	256	492
7	168	484
8	236	692
9	268	700
10	100	292
11	168	472
x	188.4	476.7

Specimens 1 - 9 were from normal individuals. Specimens 10 & 11 were from patients with beta-thalassaemic trait.

In the agarose gel electrophoresis (Fig. 4), DNA extracted by the 'Buffy-coat' method (lanes 2, 4, 6 and 8) exhibited a higher intensity of fluorescence compared to DNA extracted by the 'Dextran' method (lanes 1, 3, 5 and 7).

DISCUSSION

The 'Buffy-coat' method yielded a mean concentration of DNA of 476.7 ug/ml (range: 212 to 700 ug/ml) whereas the 'Dextran' method yielded 188.4 ug/ml (range: 64 to 340 ug/ml) (Table 1). This difference was confirmed by the observations in the agarose gel electrophoresis.

Although the 'Buffy-coat' method is more tedious compared to the 'Dextran' method, it gave a higher yield of DNA for the same amount of blood used. The reason could be that in the 'Buffy-coat' method, less leucocytes were trapped in the RBC thus allowing more leucocytes to be collected.

The 'Buffy-coat' method is an acceptable alternative method for isolation of human leucocytes when dextran is unavailable or when the leucocyte count of the individual is low.

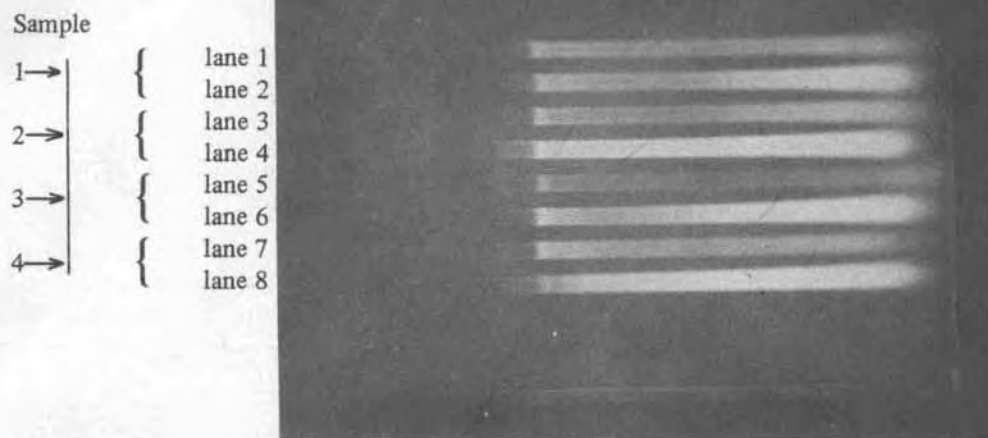


FIG. 4: Agarose gel electrophoresis of 4 DNA samples extracted by 'Dextran' and 'Buffy-coat' method respectively.

ACKNOWLEDGEMENT

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