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Full Length Research Paper

Pilot study of DNA extraction from archival unstained bone marrow slides: comparison of three rapid methods

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DNA can be isolated from a variety of human sample sources including anti-coagulant whole blood, bloodstains, hairs, tissue samples and buccal epithelial cells. The purpose of this study was to compare yield and quality of DNA samples obtained with the use of three different methods. The ability of these procedures to provide DNA for polymerase chain reaction (PCR) amplification from archival unstained bone marrow slides was tested on 35 different patients' slides. Boiling in distilled water (A), proteinase K/Tween 35 method coupled with simplified phenol/chloroform isoamyl alcohol protocol (B) and modified commercial nucleon extraction and purification protocol (C, Amersham Life Science) gave extraction efficiencies of 57, 74 and 100% respectively. Our results demonstrate that rough DNA extraction methods have decreased efficiencies compared to complete DNA extraction protocols and that the latter are required to ensure highly reproducible results from archival unstained bone marrow slides.

Key words: DNA, polymerase chain reaction, bone marrow slides, reagent kit.

INTRODUCTION

During the past few years the polymerase chain reaction (PCR) has become a major research and diagnostic technique in medicine. An attractive future of PCR is that, unlike other molecular techniques, high quality DNA is not required for successful analyses of clinical specimens. Since minute quantities of degraded DNA can serve as the template for the reaction, the method is ideally suited to a template extracted from archival clinical specimens (Poljak et al., 1995). The combined advantage of exquisite sensitivity and the ability to use routinely processed archival materials allow large-scale retrospective studies to be carried out (Pabst et al., 1996).

Unstained or Giemsa-Stained glass slide smears of cells from bone marrow aspirates or peripheral blood are common archival material available in many hematology departments. Some previous studies have shown the possibility of extracting amplifiable DNA and RNA from archival air-dried unstained bone marrow slides (Fey et al., 1987; Grünewald et al., 1991; Pabst et al., 1996), as well as from archival Giemsa-stained peripheral blood smears (Kimura et al., 1995; Yokota et al., 1995; Schoch et al., 1996), saliva, virginal and postcoital smears (Dino-Simonin et al., 1997), smears of tissue fluid and inflammatory exudates (Alger et al., 1996), archival cytogenetic slides (Sago et al., 1996) and archival Giemsa-stained bone marrow slides (Vince et al., 1998). To the best of our knowledge, 3-6 reports concerning successful extraction of DNA from archival unstained bone marrow slides have been published (Fey et al., 1987; Grünewald et al., 1991; Pabst et al., 1996). However, a cumbersome and laborious 2-3 extraction protocol with the hazardous organic solvents phenol and chloroform is necessary for this purpose.

For large-scale routine processing of archival material, DNA extraction should be simple and rapid, and must not affect the PCR amplification. Moreover, as few steps as possible must be involved to minimize the possibility of contamination. Therefore, in the present study we

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evaluated the DNA extraction efficiencies using three rapid DNA extraction protocols, which take less than two hours in order to find the most suitable method for routine processing of unstained bone marrow slides. In addition, the purpose of this study is to extract DNA from archival bone marrow slides for retrospective analysis such as mutational analysis of tyrosine kinase receptors in leukemia.

MATERIAL AND METHODS

Samples

35 routinely-processed unstained bone marrow slides (17 patients with acute lymphplastic leukemia and 18 patients with acute myeloid leukemia), which had been stored in a box at room temperature in an air-conditioned storage room for up to 15 years (1987 to 1995) in the archives of Hematology Laboratory at king Abdulaziz University Hospital, Jeddah, were included in the study. Before DNA extraction, the slides were examined for any glass cracking. For study purposes, slides were subsequently divided into three groups, each consisting to 35 slides from each patient.

DNA extraction

Bone marrow smears were scraped off the glass slides in a laminar flow hood with a sterile scalper blade and the resulting powdered material was transfused to 1.5 ml tubes and processed according to the following three protocols:

Protocol A: The scraped material was resuspended in 100 µl of sterile double-distilled DNAse, RNAse free water, boiled for 20 min, then centrifuged for 5 min, and the supernatant was discarded. 100 µl of sterile double-distilled DNAse, RNAse free water was added to the pellet and the tube was left on rotator to dissolved DNA for 2 h.

Protocol B: The procedure used was that of Poljak and Barlic (1996). Briefly, the scraped material was resuspended in 200 µl of digestion buffer (50 mM Tris HCL, 1 mM EDTA and 1% Tween-20) containing 800 µg/ml of proteinase K and then incubated at 56°C for 1 h. After digestion was complete, 200 µl of phenol : chloroform saturated with 10 mM Tris-HCL (pH 8.0) was added to each tube and shaken vigorously for 1 min followed by centrifugation at 6000 g for 10 min. After centrifugation, the supernatant (which contain DNA) transferred into another tube; the DNA was precipitated with 2 volume of cold absolute ethanol at -20° C for 40 min. After centrifugation at 6000 g for 5 minutes, the supernatant was discarded and the precipitate (DNA) was air dried and re-dissolved in 50 µl of sterile double-distilled DNAse, RNAse free water.

Protocol (C) nucleon: Genomic DNA was extracted from the scraped material of each slide using the Nucleon™ BACC1 DNA extraction kit (Nucleon Biosciences). The scraped material was mixed with 1 ml of lysis buffer (solution A; 10 mM Tris-HCl, 320 mM sucrose, 5 mM $MgCl_2$, 1% Triton X-100, pH 8.0) in 1.5 ml tube for 5 min at room temperature. A cell pellet was collected by centrifugation at 3000 g for 4 min and the supernatant removed. The cell pellet was washed in a further 1 ml of lysis buffer and centrifuged at 1500 g for 5 min. The cell pellet was resuspended in 350 µl of nuclear lysis buffer (solution B; 400 mM Tris-HCl pH 8.0, 60 mM EDTA, 150 mM NaCl, 1% SDS). The sample was then deproteinized by adding 100 µl of 5 M sodium perchlorate. After inverting the tube seven times, the solution was transferred to 1.5 ml micro centrifuge tube. 600 µl of pre-cooled chloroform was added and the solution mixed for 5 min or by inverting the tube at least seven times. The solution was centrifuged at 1200 g for 1 min and 150 µl of Nucleon Silica suspension was added on top of the solution. Following centrifugation at 1300 g for 3 min, the upper aqueous layer was removed into a 1.5 ml tube containing 900 µl of ice-cold absolute ethanol, to precipitate the DNA. After gently inverting, centrifugation for 5 minutes at 1500g and discarded the supernatant then added 1 ml of 70% ethanol, followed by centrifugation for 5 min at 1500 g, and then discarded the supernatant. The precipitated was air dried and redissolved in 50 µl of sterile water to dissolve DNA. DNA samples were quantified by optical density measurement and then stored at –20°C until the use.

Determination of DNA concentration

Extracted DNA from all methods was determined by quantitative method based on the optical density measurement to assess the purity of extracted DNA. The concentration of DNA in solution was measured by reading the absorbance at a wavelength of 260 nm. Samples were diluted 1:100 with sterile water in a 1.5 ml tube and transferred to a 1 ml quartz cuvette.

PCR Amplification of extracted DNA

The quality of DNA preparation was checked by polymerase chain reaction (PCR) amplification of a segment of human factor V (FV) gene (154 bp) using two oligonucleotide primers as described by Kirschbaum anf Foster (1995). All PCR reactions comprised the following; 500 ng of genomic DNA, 16.6 mM-$(NH4)_2SO_4$, 67 mM Tris HCL (PH 8.0), 10 mM B-mercaptoethanol, 100 µg bovine serum albumin (BSA), 300 ng of each primer, 200 µM dNTPs, 1.50 mM $MgCl_2$, and 1 U Taq DNA polymerase in a final volume of 50 µl. Samples were initially denatured at 94°C for 5 min. DNA amplification was performed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 30 s and extension at 72°C for 30 s. 5 µl of each PCR products was loaded onto 5% polyacrylamide gel to ensure the amplification had occurred. PCR products were visualized by UV illumination following ethidium bromide staining. PCR was scored positive when a band of appropriate size was visualized and negative when no band could be seen.

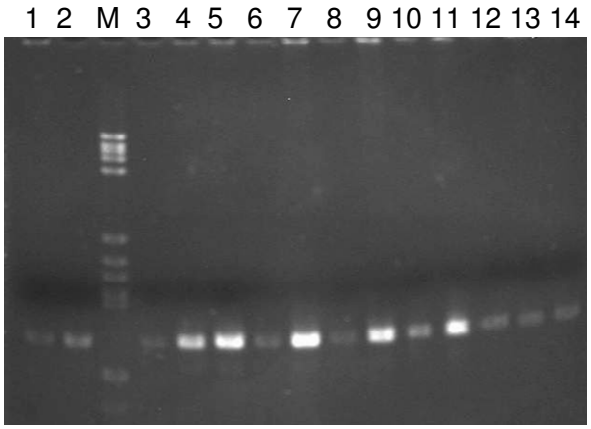

Figure 1. Protocol B results of ethidium bromide stained polyacrylamide gel showing PCR amplification of a fragment of human factor V gene in most samples with the expected 154 bp fragment. M, PBR322/HaeIII DNA marker.

Table 1. DNA produced by the three different protocols; boiling in distilled water (A), proteinase K/Tween 35 method coupled with simplified phenol/chloroform isoamyl alcohol protocol (B) and modified commercial nucleon extraction and purification protocol (C).

Sample number	Protocol A		Protocol B		Protocol C	
	Concentration (µg/ml)	260/280 ratio	Concentration (µg/ml)	260/280 ratio	Concentration (µg/ml)	260/280 ratio
1	1420	0.969	986	1.129	847	1.69
2	1370	0.948	310	1.148	428	1.532
3	515	0.989	260	1.040	203	1.667
4	1895	1.026	1225	1.150	1487	1.64
5	1775	1.030	2115	1.119	1621	1.56
6	269	0.986	255	1.159	193	1.274
7	1420	0.969	350	1.129	306	1.862
8	1370	0.948	310	1.148	263	1.473
9	635	0.989	260	1.040	179	1.658
10	3470	0.994	950	1.098	827	1.510
11	2495	0.967	2880	1.083	1930	1.634
12	1000	0.948	370	1.028	298	1.758
13	1245	0.940	1180	1.049	921	1.889
14	835	0.981	355	1.076	309	1.806
15	890	1.108	1310	1.031	632	1.710
16	1270	0.934	570	0.999	601	1.794
17	1485	0.958	1335	1.064	1103	1.686
18	1325	1.026	1225	1.150	1043	1.759
19	775	1.030	615	1.119	472	1.532
20	660	0.986	255	1.159	202	1.667
21	1425	0.969	350	1.129	321	1.850
22	1370	0.948	310	1.148	293	1.553
23	825	0.989	260	1.040	285	1.174
24	1070	0.994	950	1.098	845	1.626
25	1295	0.967	880	1.083	758	1.473
26	1000	0.948	370	1.028	390	1.658
27	1245	0.940	1180	1.049	1065	1.510
28	375	0.981	355	1.076	289	1.634
29	1090	1.081	1310	1.091	745	1.758
30	1270	0.934	570	0.999	1270	1.889
31	1485	0.958	1335	1.064	1280	1.806
32	895	1.011	615	1.060	489	1.710
33	1050	0.967	1105	1.038	834	1.794
34	540	0.961	325	0.999	432	1.686
35	1360	0.965	1075	1.080	1056	1.655

RESULTS AND DISCUSSION

The concentration of extracted DNA from each protocol was determined by using DNA calculator (Amersham) and the purity of DNA then was evaluated by comparing at the absorbance ratio of 260 and 280 nm. The ratio of 1.7-2.0 indicate that the pure DNA concentration. Table 1 summarized DNA concentration that extracted by each method including the absorbance ratio of 260/280.

The overall results obtained by the PCR amplification of 154 bp of FV genes on DNA extracted by three rapid methods from 35 archival unstained bone marrow slides differ. DNA produced by the simplest method (protocol A) was amplified only in 20 (57%) of the total slides (figure not shown). In contrast, the second method (protocol B) produced amplifiable DNA in 26 (74%) of the 35 unstained slides (Figure 1). Finally, in the last method (protocol C), the amplified DNA appeared in all of the 35 unstained slides' preparation (Figure 2). This indicates that the commercially kit gave better DNA compared to simple method.

The method used to extract DNA from archival unstained bone marrow slides appears to be the most critical parameter in determining extraction efficiency and purity. In the present study the percentage of the samples which were successfully amplified increased from 57% in

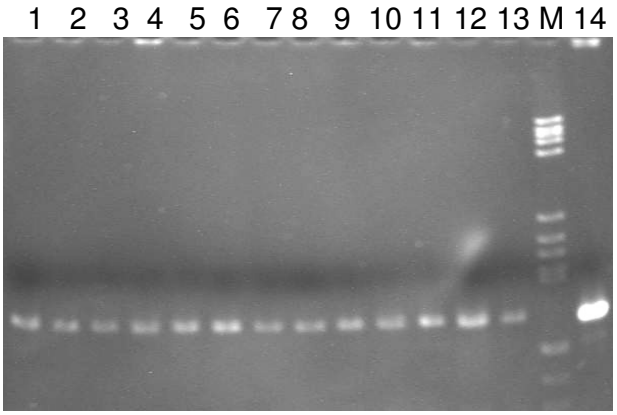

Figure 2. Protocol C results of ethidium bromide stained polyacrylamide gel showing PCR amplification of a fragment of human factor V gene in most samples with the expected 154 bp fragment. M, PBR322/HaeIII DNA marker.

samples treated by the rough boiling method to 100%, when the DNA was extracted by more sophisticated methods. Our inability to successfully amplify all DNA extraction by simpler rough methods may either reflect an inhibition of the PCR process itself by one or more compound, known or unknown to inhibit the PCR amplification (reviewed in Wilson, 1997) or an inability to extract good quality template DNA. But in protocols B and C the result or amplification efficiency was 74 and 100%, respectively.

The second reason for the dismal performance of PCR on DNA samples extracted from unstained bone marrow slides by simpler rough methods seems to be an insufficient DNA yield. Theoretically, the amount of the released target DNA can be small if the inhibitor residues (excess salts or protein) negatively interfere with cell destruction and subsequent DNA liberation from the cells, or more likely if the rough DNA extraction process

In the conclusion, our comparative evaluation of three rapid DNA extraction methods demonstrates that rough DNA extraction methods have decreased DNA extraction efficiencies compared to complete DNA extraction protocols and that the latter are required to ensure highly reproducible results from archival unstained bone marrow slides. We hope that our comparative analysis will help to improve and simplify retrospective hematological research studies on archival clinical specimens and will be of value for establishing molecular diagnosis of some hematological disorders in the cases where no frozen bone marrow or blood samples have been kept.

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