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Original Article

An Efficient Method for DNA Extraction from Paraffin Wax Embedded Tissues for PCR Amplification of Human and Viral DNA

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ABSTRACT

Background and Objective: **Formalin-fixed paraffin-embedded tissues are a valuable source of DNA for molecular studies. We designed and optimized an efficient procedure for DNA extraction from formalin-fixed paraffin embedded tissues.**

Materials and Methods: **Seventy three blocks of cervical paraffin-embedded tissues were investigated. DNA was extracted using 45 minutes boiling in alkaline solution together with 10 beads of Chelex-20, followed by phenol-chloroform extraction and alcohol precipitation.**

Results: **This method produced DNA suitable for amplification using primers specific for human SMN and β globin genes in 98.63% and 82.2% of samples respectively. We also detected human papillomavirus DNA in 58.33% of appropriate samples.**

Conclusion: **This procedure provides simple and efficient method for recovery of amplifiable genomic and viral DNA from paraffin wax embedded tissues.**

Key words: DNA, Paraffin Embedding, Polymerase Chain Reaction

Introduction

The most common human archival specimens are formalin-fixed, paraffin-embedded tissues (PETs). Paraffin wax embedded tissues are a remarkable source for molecular studies due to the accessibility of large pathology archives of tissues related to clinical cases in almost all hospital-affiliated pathology departments. The polymerase chain reaction (PCR) has confirmed to be a rapid and particularly sensitive method for examining DNA from PETs. An adequate storage of samples and undertaking a procedure to produce high-quality extracted DNA are important factors for obtaining successful results from PCR. DNA can be extracted from PETs, but this is usually unsuitable for most molecular techniques

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which require high molecular weight genomic DNA. This unsuitability could result from a) The degradation of DNA, which may results from the type of fixative solution used, the storage conditions, the suppliers, and the long time between surgical tissue removal and fixation; b) The fragmentation of nucleic acids due to formalin fixation (1); and c) The absence of a detectable amount of target DNA in the small tissue samples or biopsy specimens (2). However, short DNA segments are also useful as a template for PCR (3).

A wide variety of methods exist for the recovery of DNA from formalin-fixed, paraffin embedded tissues and it is interesting that sometimes despite using same methods different results were obtained in different studies (4-6). The technical procedures for extraction of DNA from these tissue sections include many steps such as deparaffinization in xylene, enzymatic digestion or other chemical treatment coupled with enzyme digestion. These methods are time consuming and involve repeated manipulations.

We evaluated different methods in our laboratory and compared them in order to define a suitable DNA extraction protocol from paraffin-embedded tissues. In this paper we explain the most efficient one which is simple, effective and relatively cheap and can be used for extraction of viral and genomic DNA.

Materials and Methods

Tissue samples

Seventy three blocks of formalin fixed, paraffin wax embedded cervical tissues prepared during the years 2000-2006 for routine histological study of cervical neoplasia were obtained from Shahid Beheshti hospital (Isfahan, Iran).

DNA extraction

DNA was extracted from 10 µm sections of paraffin wax embedded tissues. Five sections were cut with standard microtome from every paraffin wax block and transferred into a 1.5 ml microtube. To prevent cross contamination between the samples, the microtome blade was washed with xylene and ethanol after sectioning of each block. Six hundred microliters of 1% SDS and 0.1 M NaOH solution (pH 12.7) (7) and 10 beads of Chelex 20 were added to each microtube. The microtubes were heated at 100 ºC in a water bath for 45 min. A cooling time of 5 min was allowed after heating. To withdraw the DNA solution, the top solidified wax layer was pierced by a micropipette tip. The solution beneath wax layer was withdrawn and transferred to a clean 1.5 ml microtube. The Chelex beads were not removed. Further steps of extraction and purification were performed according to the Shi et al (8).

DNA purity, yield and size

DNA purity was assessed with a spectrophotometer and calculated by ratio of the DNA optical density (OD 260) and protein optical density (OD 280). DNA yield was calculated from DNA optical density (OD 260) for clean DNA samples. DNA size was analyzed by electrophoresis pattern of sample aliquots (5 µl) in a 1% agarose gel stained with ethidium bromide and visualized under ultraviolet light.

Genomic DNA PCR

PCR amplification of genomic DNA was performed using primers specific for human SMN (9) and β globin genes (Table 1). PCR reaction mixture contained 1U Taq polymerase, 1 X PCR buffer (50 mM KCl and 10 mM Tris-HCl (pH 8.4), AMS buffer containing 20 mM ammonium sulfate, 75 mM Tris-HCl (pH 8.8)), 0.2 mM of each dNTPs, 1.5 mM $MgCl_2$, 10 pmol of each primers and 100-200 ng of extracted DNA. The PCR conditions were as follows: denaturation at 94 °C for 5 minutes, followed by 30 cycles of denaturation at 94 °C for 1 minute, annealing at 55 ºC for SMA primer and 63 ºC for β globin primer for 1 minute and extension at 72 °C for 1 minute followed by 5 minutes final extension at 72 °C. Purified DNA from nucleated cells of whole blood was used as positive control and sterile distilled water was used as negative control. The PCR amplification products were run on 2% agarose gel, stained with 0.5 µg/ml ethidium bromide and visualized under ultraviolet light.

The samples yielded positive results with SMA primers were further analyzed for presence of human papillomavirus (HPV) DNA. HPV was amplified with the L_1 consensus primers Gp5+/Gp6+ (Table 1) (10). The reaction mixture was the same as for the SMN and β globin genes except for $MgCl_2$ concentration which was increased double-fold. The cycling conditions were initial denaturation at 94 °C for 5 minutes, followed by 40 cycles of denaturation at 94 °C for 1 minute, annealing at 55 °C for 2 minutes, extension at 72 °C for 2 minutes and a final extension step at 72 °C for ten minutes. A standard HPV DNA was used as positive control, and sterile distilled water as negative control. The amplicons were electrophoresed using 2% agarose gel stained with ethidium bromide and visualized under ultraviolet light.

Table 1: Oligonucleotide primers used for viral and genomic DNA amplification

Primer	Sequence (5′ 3′)	Target region	Size of PCR product (base pairs)
SMAR11 SMAX7DRA	AGACTATCAACTTAATTTCTGATCA CCTTCCTTCTTTTTGATTTTGTTT	SMN gene	185
IVSI-1-N B	TTAAACCTGTCTTGTAACCTTGATACCC ACCTCACCCTGTGGAGCCAC	β globin gene	281
$GP5^+$ $GP6^+$	TTTGTTACTGTGGTAGATACTAC GAAAAATAAACTGTAAATCATATTC	L_1 region of HPV	150

Results

Measurement of DNA yields and purity assessment

Electrophoretic pattern

Fig 1 shows the electrophoretic pattern of extracted DNA. Significant amounts of high molecular weight DNA was evident.

PCR analysis

The 185-bp fragment of the SMN gene was amplified in 72 out of 73 cases (98.63%) (Fig. 2) and amplification of 281-bp fragment of the β globin was successful in 60 out of 73 cases (82.2%) (Fig.3). PCR amplification of SMA positive samples for HPV genome resulted in 58.33% positive cases. The amplicon size for HPV was 150bp (Fig. 4).

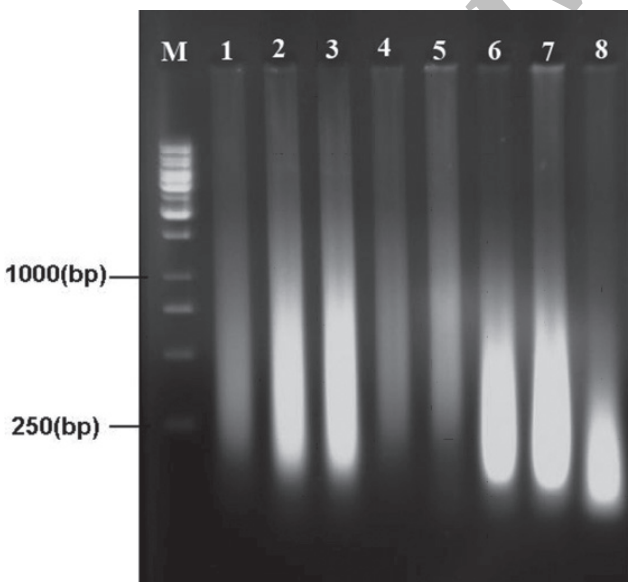

Fig. 1: Electrophoretic patterns of DNA extracted from paraffin-embedded tissue sections of cervix. M: 1 Kb marker.

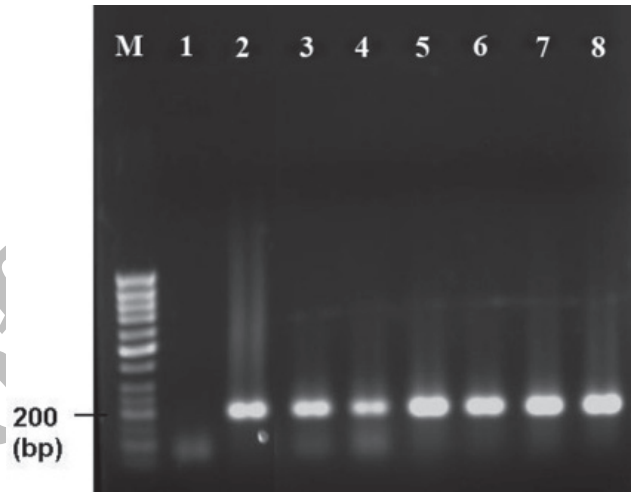

Fig. 2: Gel electrophoresis amplification products of 185bp fragment of the SMN gene sequence. M: 50bp marker. Lane 1: negative control; lane 2: Positive control; lanes 3-8: positive samples.

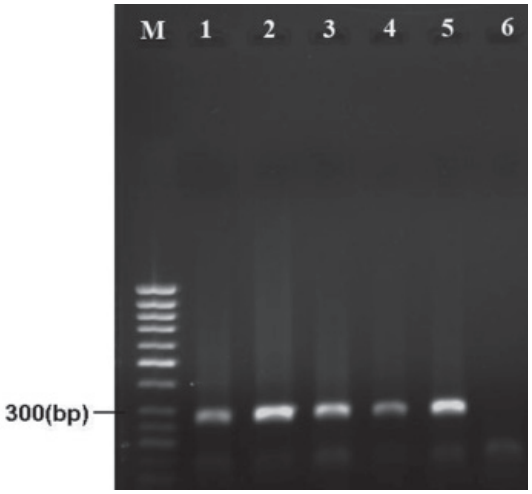

Fig. 3: Gel electrophoresis amplification products of 281bp fragment of the β globin gene sequence. M: 50bp marker. Lane 5: positive control; lane 6: negative control; lanes 1-4: positive samples.

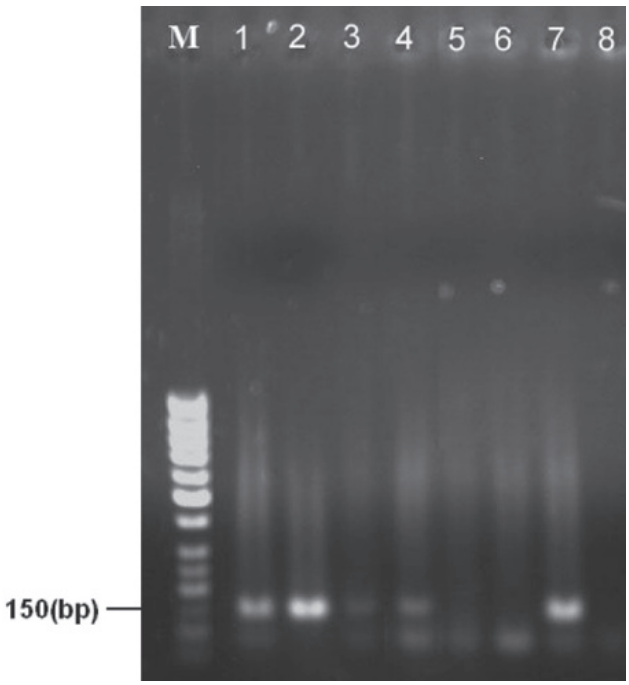

Fig. 4: Gel electrophoresis amplification products of 150bp fragment of the L_1 open reading frame (ORF) of HPV. M: 50bp marker. Lane 7: positive control; lane 8: negative control; lanes 1-4: positive samples; lanes 5, 6: negative samples.

Discussion

There are a large number of methods for recovery of DNA from formalin-fixed paraffin-embedded specimens. Some authors believe the main obstacle in preparing DNA suitable for PCR amplification is removal from paraffin wax (4). Stanta et al believe the removal of paraffin is important (11) and it otherwise leads to PCR inhibition during subsequent PCR. Despite this, some authors used no specific steps to remove the paraffin because they believe either its removal is unnecessary or paraffin omission takes place during tissue processing (6;7;12). The most common technique to remove paraffin is based on use of xylene and ethanol washes (13;14). This method that was described by Goelz et al (15) in 1985 is time consuming and requires multiple steps. Alternative method includes removal of paraffin through melting using microwaves or thermal cycler (16). Following any pretreatment that may have been applied, a number of methodological variants have been published for subsequent DNA recovery and purification. Most of these techniques, involve use of digestion buffer containing Tris-HCl, EDTA, detergents and proteinase K followed by use of phenol/chloroform, simple boiling or Chelex for purification.

In this study, we used 0.1 M NaOH and 1% SDS solution (7) as digestion buffer. Due to its simple composition, it is easily prepared in any laboratory. In this regard, NaOH plays a critical role in disruption of cell membrane for solubilization of proteins (17). Boiling duration was 45 minutes in our study; through that retrieval solution containing tissue sections become pellucid. We did not use xylene or other similar solvents because the step of deparaffinization was omitted due to thermal melting of the wax in hot solution. No difference observed when xylene and ethanol or heat treatment was used for deparaffinization (data not shown). Therefore, application of organic materials could safely eliminate deparaffinization and hence a reduction in steps of extraction using this method. Furthermore, we used Chelex 20 beads in our experiment. Sample processing by Chelex has been successfully employed for paraffin wax embedded tissues (18;19). The effect of Chelex, which prevents the degradation of DNA, is thought to be due to chelating metal ions which may act as catalysts in the breakdown of DNA at high temperatures in low ionic strength solutions (20;21). It is also possible that Chelex could bind to potentially PCR inhibitor substances and therefore facilitate PCR amplification (21). Coombs et al (4) achieved better result using Chelex. Based on our results, a 281bp fragment of β-globin gene was amplified in 82.2% of our samples. This result was significantly higher than the result reported by lamballerie et al (53.3%) (22) that used xylene treatment and Chelex and that of coombs et al (61.3%) (4), who used thermal cycler and Chelex for deparaffinization and purification respectively. An additional advantage of our developed method was simultaneous recovery of high quality viral DNA.

Taken together, other than procedure we used for extraction of DNA, several other factors impress quality of nucleic acids within PETs. These include pre-fixation factors (e.g. tissue type and amount, degree of autolysis); fixation related factors (e.g. pH, temperature, and duration of fixation, as well as the type of fixative); and post-fixation factors (e.g. temperature and duration of storage) (23-25). This may in part explain the underlying reason that how different results were obtained using same protocols in different studies.

Conclusion

Using the method described here, it is possible to obtain PCR compatible DNA (genomic as well as viral) from paraffin wax embedded cervical tissues. PCR product up to 281bp in size was consistently generated, which in the majority of cases permits further genetic analysis to be performed.

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