High yield gold nanoparticle-based DNA isolation method for human papillomaviruses genotypes from cervical cancer tissue samples

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Abstract: Gold nanoparticles (AuNPs) are commonly used in biosensors of various kinds. However, its application to extract DNA from cancer tissues has not been extensively studied. The purification of DNA from cancer tissues is an important step in diagnostic and therapeutic development. Almost, all cervical cancer cases are associated with high-risk human papillomavirus (HR-HPV) infection. Accurate viral diagnosis has so far relied on the extraction of adequate amounts of DNA from formalin-fixed, paraffin-embedded (FFPE) tissue samples. Till now, no specific and sensitive DNA purification method has been introduced for the extraction of HR-HPV from FFPE tissue. Since the commercially available purification kits are not sensitive and specific enough for HR-HPV DNA targets, in this study, a DNA purification method was designed based on AuNPs to purify sufficient amounts of HR-HPV DNA from cervical cancer tissue samples. AuNPs were coated with a series of oligonucleotide probes to hybridize to specific DNA sequences of HR-HPV genotypes. Results showed that 733 out of 800 copies of type-specific HPV DNA were recovered with complete specificity, compared to 36 copies with a standard commercial kit (Qiagen FFPE). The high yield of DNA (91.6%) is the main advantage of the AuNPs-probe purification method.

1 Introduction
Gold nanoparticles (AuNPs) have advantages in terms of analytic function [1, 2], optical and electronic detectability that makes them useful in a wide variety of biosensors and therapeutic applications [3–7]. In particular, the attachment of oligonucleotide probe sequences to AuNPs (Oligo-AuNPs) could serve as high-affinity sensors for the extraction of DNA in bacteria-based infections [8], parasitic diseases [9], viruses [10], and cancer biomarkers [11, 12]. Among women, cervical cancer with a high incidence rate is significantly associated with high-risk human papillomavirus (HR-HPV) [13, 14]. To investigate the role of HPV in cervical cancer, archived tissues from diagnostic pathology laboratories are valuable resources [15]. Many such samples are preserved in the form of formalin-fixed, paraffin-embedded (FFPE) tissue, the standard method of preservation for many years [16]. FFPE treated samples are beneficial in immunohistochemistry and haematoxylin and eosin staining. However, they are difficult to be analysed by molecular diagnostic methods such as microarray analysis and quantitative polymerase chain reaction (qPCR), which results from significant damage to nucleic acids and formaldehyde-induced cross-linking in FFPE processing [17, 18].

To extract nucleic acid from FFPE tissues, paraffin has to be removed or melted [19, 20], giving rise to highly variable yields and quality of the extracted nucleic acid, which is highly dependent on the sample age, fixation condition, and size of tissue [21, 22]. Several methods and protocols have been developed to help extract nucleic acids from FFPE blocks more efficiently, and to prepare it for downstream analyses [23–25]. Nearly all commercially available FFPE sample preparation and purification kits are designed to extract total RNA or DNA from the tissue and several methods have been proposed to overcome problems experienced during these procedures [20]. Recently, the procedure of 69 commercial kits from different companies designed specifically for DNA and/or RNA extraction from FFPE sections have been reviewed [26]. However, any kits might have different strengths and weaknesses in terms of DNA yield, purity, and quality. We chose instead to develop a method to extract specific target sequences from such tissue, to increase the reliability, accuracy, and sensitivity of polymerase chain reaction (PCR) analysis. While HPV DNA can be specifically detected and amplified for diagnostic purposes using Oligo-AuNP probes [27, 28], to our knowledge, no protocols have been previously reported for the type-specific extraction of HPV DNA from FFPE tissue.

2 Experimental procedures
2.1 Materials
pHPV-16 (ATCC® 45113D) and HPV-18 (ATCC® 45152D) purified plasmid DNAs were purchased from American Type Culture Collection (ATCC; USA). Plasmid vector pBME containing a synthetic partial DNA sequence of the L1 open reading frame (ORF) of HPV genotypes 45, 52, and 58 was purchased from Biomatik (Canada). Chloroauric acid, trisodium citrate, and sodium borohydride were purchased from Sigma (Germany). TA cloning vector pTZ57R and all restriction digestion enzymes were purchased from Thermo Fisher Scientific Inc. (Lithuania). Primers and probes were purchased from Metabion AG (Germany). SYBR Premix ExTaq II (Tli RNaseH Plus) was purchased from Takara (Japan). GF-1 plasmid DNA extraction kit and GF-1 Gel DNA recovery kit were purchased from Vivantis (Malaysia). QIAamp DNA FFPE tissue kit was purchased from Qiagen (Germany). Ampliquality HPV-TYPE kit was purchased from AB Analitica (Italy). All other chemicals were purchased from Merck (Germany).
2.2 Instruments
A ultraviolet (UV)/Visible (Vis) Spectrophotometer (WPA Lightwave II/Biochrom, US/UK) was used to determine the UV–Vis absorption and to determine the range of amplicon concentration. Hydrodynamic diameter (HD) of the particles was confirmed by dynamic light scattering (DLS) instrument (Nanoflex, Microtrac, USA) and zeta potential was determined by Nano-ZS Zetasizer. Transmission electron microscopy (TEM) image was captured by Zeiss-EM10C-80 kV (Zeiss, Germany) and particle size analyses were conducted using ImageJ software version 1.47v, an image analysis software developed by the NIH (http://imagej.nih.gov/ij/). The amplification of extracted DNA was performed on a Rotor-Gene Q 5plex Platform real-time PCR system (Qiagen, Germany) and data analysis was performed by Rotor-Gene Q Series Software (Qiagen, Germany/version 2.0.2).

2.3 Methods

2.3.1 Plasmids and bacterial strains: HPV-16 and HPV-18 purified plasmid DNAs were received in Escherichia coli strain DH5α. A synthetic partial DNA sequence of the L1 ORF of HPV genotypes 45, 52, and 58 containing designed restriction endonuclease recognition sites (BamHI, HindIII, and NdeI) were also received in plasmid vector pBME in IET Nanobiotechnol. 556 and purified plasmid DNAs were received in plasmid as a source of HPV-31 and 33 DNA in our experiment. DH5α. A synthetic partial DNA sequence of the L1 ORF of HPV

2.3.2 Primers and probes design: GP5+/GP6+ consensus primers were used to amplify to a conserved region of the HPV L1 gene [29]. Using Gene Runner software, oligonucleotide probes were designed to specifically hybridise to their target sequence of each genotype (Table 1). The DNA probes were prepared with a thiol linker group (HS–(CH2)6–) to conjugate to the AuNPs surface.

2.3.3 Synthesis of AuNPs: AuNPs were synthesised as previously described [30]. Briefly, chloroauric acid (0.5 ml of 0.01 M) was dissolved in 18 ml ultra-purified water, sodium citrate (0.5 ml) in the concentration of 0.01 M was then added, and the mixture was incubated at 5 min at room temperature. Sodium borohydride (0.5 ml of 0.1 M) was rapidly injected into the reaction mixture to obtain particles <10 nm. Particles were then stored at 4°C in a dark place.

2.3.4 Conjugation of oligonucleotide probes to AuNPs: Thiol-modified oligonucleotide probes conjugation was performed through the reported procedure [31]. Initially, a series of experiments in hybridisation was performed to optimise the concentration of thiol-modified DNA probes and AuNPs solution (Fig. 1). Indeed, different concentrations of the thiol-modified probe (5, 10, 20, and 40 µM) were separately added to the solution (1.06 µg/ml, 980 µl) of AuNPs and incubated in a water bath for 24 h at 37°C. Besides, all the thiol-modified probe concentrations were also added to a concentration of 2.13 µg/ml AuNPs solution. For strong attachment of probes to the surface of AuNPs, salt buffer solution (0.05–1.0 M sodium chloride (NaCl) in 10 mM NaHPO4, pH 7.4) at different concentration was added to the mixture. The mixture was centrifuged for 30 min at 19,530 g to remove unbounded probes from the functionalised AuNP solution. Following the removal of the supernatant, the red oily precipitate was washed twice with phosphate buffer saline (PBS, pH 7), followed by centrifugation and redispersion in 0.05 M NaCl solution. Moreover, 20 and 40 µl volume of optimum concentration from the functionalised AuNPs solution was tested to achieve the final assay volume.

2.3.5 Optimisation of hybridisation temperature and time: Using AuNP to achieve maximum efficiency, different hybridisation conditions were optimised for the purification of HPV DNA from FFPE tissue samples. To establish the optimal hybridisation time, the functionalised AuNPs solution was allowed to react with the PCR products for times ranging from 30, 45, and 60 min at a pre-determined temperature, which was ∼5°C below the melting temperature.

2.3.6 Preparation of template DNA for hybridisation: A ten-fold dilution series range 10¹⁰–10⁰ copies of plasmids containing high-risk HPV DNA were PCR amplified by GP5+/GP6+ consensus primers to determine the method ability to purify the lowest concentration of HPV DNA.

The reaction mixture (final volume 25 µl) consisted of 12.5 µl of SYBR Green Premix, 2 µl of the template DNA, and 0.4 µM of each primer. The amplification was performed on the real-time machine at 95°C for 30 s, followed by 50 cycles of 95°C for 5 s, 60°C for 20 s, and 72°C for 20 s. Melting curve analysis of the amplified products was conducted at the end of each PCR to confirm that one PCR product was amplified.

2.3.7 Purification method for high-risk HPV DNA: To evaluate our newly developed purification method for the yield and purity of DNA, a volume of 20 µl of the probe–AuNP conjugate was mixed with 2 µl of a dilution series (ten-fold step) of PCR products and 8 µl of 2× saline-sodium citrate buffer. The hybridisation reaction was performed at 94°C for 12 min followed by 30 min incubation of HPV-16 at 57°C, HPV-18, 31, and 33 at 45°C, HPV-45 and 52 at 56°C and HPV-58 at 54°C.

To remove excessive unbounded DNA, the tubes were centrifuged at 16000 g for 10 min. Following supernatant removal, the red oily precipitate was then washed once with PBS containing 0.01% Tween 20 and twice with PBS (pH 7.2–7.6 at 25°C), and centrifuged at 16,000 g for 10 min and the pellet was dispersed into 20 µl double distilled water.

DNA bound to the probe–AuNP conjugate was eluted by heat treatment. Eppendorf tubes were heated at 95°C for 2.5 min and then centrifuged at 16,000 g for 10 min. The supernatant was transferred to a fresh tube and used directly in the PCR assay.

2.3.8 SYBR green-based quantitative real-time PCR: The DNA purification method accuracy was investigated by

| Table 1 Specific probes designed for the specific purification of HPV DNA |
|---------------------------------------------------------------|
| **Probe names** | **Probe sequence 5'-3’** |
| HPV-16 probe | TTGTCACTTGTTGCTAGCATACGCTTTTTTTTTTTTTTT-thiol-C6 |
| HPV-18, 31, 33 probe | TTATTTTGATTGCTAGTGATACCTTTTTTTTTTTTTTT-thiol-C6 |
| HPV-45 probe | TTGGTGTTACTGCTACTGACTACCTTTTTTTTTTTTTTT-thiol-C6 |
| HPV-52 probe | TTGTGACAGTTGCTGGATACACTACCTTTTTTTTTTTTTTT-thiol-C6 |
| HPV-58 probe | TTATTTGTTACCGTGTTGATAACCTTTTTTTTTTTTTTT-thiol-C6 |

Bold and underline sequences are complementary to the sequence of the HPV, poly T at the 3’-terminus acts as a spacer for increasing the distance between probes and surface of AuNPs.

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quantitative real-time PCR using GP5+/GP6+ consensus primers and Takara SYBR Green Master Mix as described above, except that a unit of Taq DNA polymerase and bovine serum albumin (BSA; 700 ng/µl) was added to enhance PCR amplification yield. For quantification of HPV DNA and to generate standard curves, in separate experiments, a real-time PCR assay was performed on tenfold dilution series of each plasmid DNAs containing high-risk HPV genotypes 16, 18, 31, 33, 45, 52, and 58 (ranging from 10^10 copies to 1 copy per reaction).

2.3.9 Sensitivity evaluation of DNA extraction methods: To determine the sensitivity of the DNA extraction methods, HPV-16 DNA containing plasmid was initially amplified by PCR assay using GP5+/GP6+ consensus primers and the amplicons concentration was determined. Following, 8 × 10^2 to 8 × 10^4 copies/µl of the amplicons were added to 200 µl of HPV DNA negative tissue lysate, and the tubes were subjected to DNA purification by AuNPs method and Qiagen FFPE DNA extraction kit. The real-time PCR assay was then performed to evaluate the sensitivity of each extraction method. The experiments were repeated thrice.

2.3.10 DNA purification from FFPE cervical tissue samples: To evaluate the efficiency of the present DNA purification method on FFPE tissue samples, a total of nine FFPE cervical cancer tissue specimens were obtained from the archive of pathology, Namazi Hospital, Shiraz, Iran. The duration of formalin storage for the clinical samples used in this study was more than 6 months. Eight 10-μm thick sections were cut and placed in a 1.5 ml microcentrifuge tube and were subjected to purification. Briefly, 180 µl of lysis buffer containing 10 mM NaCl, 1 mM EDTA, 20 mM Tris–HCl, and 10% sodium dodecyl sulphate in pH 8.0, was added directly to the paraffin sections and incubated for 20 min at 120°C. Melted paraffin wax was then mixed to obtain a homogeneous suspension in lysis buffer. Tubes were briefly centrifuged to bring any condensation down from the walls and lid. Proteinase K at a concentration of 10 mg/ml was added to the tubes and incubated at 65°C for 16 h in the heat block. Tissue lysate was then adopted as the target DNA to perform the proposed purification strategy as shown in Fig. 2.

DNA extracts were tested using a quantitative real-time PCR assay, and its concentration was calculated from the standard curve. The HPV genotypes were then confirmed by the Ampliclarity HPV-TYPE genotyping test, which is based on the reverse hybridisation method.

2.3.11 Specificity determination of assay: To perform the specificity of AuNPs purification method, nine HPV negative FFPE samples from benign ovarian tumour, and FFPE cervical specimens previously tested positive for the low-risk HPV genotypes 6, 53, 87, and 90 were subjected to the purification and genotyping method as described above.

3 Results and discussion

3.1 Synthesis and characterisation of Oligo-AuNPs

To begin the preparation of AuNPs bearing HR-HPV type-specific complementary oligonucleotide probes, spherical AuNPs were synthesised by co-precipitation in the presence of sodium citrate, with size-controlled by addition of sodium borohydride [30]. Synthesised AuNPs had UV–Vis spectra with a maximum peak at 510 nm. Following interaction with the oligonucleotide, the λ maximum of AuNPs changed from 510 to 540 nm (Oligo-AuNP) (Fig. 3). DLS was done to evaluate the HD of AuNPs. As shown in Fig. 4, the diameter of nanoparticles was measured dominantly from 2.16 nm up to 15.1 nm. The AuNPs concentration (C_AuNP) was estimated from particle size (d = 5.1) and absorbance (A = 0.522) using theoretical relationship of (1), with C1 = −4.75 and C2 = 0.314 [32]

\[ d = \frac{A(5.89 \times 10^{-6})}{C_AuNP \exp(C_2)} \] (1)

C_AuNP was calculated as 2.13 µg/ml.

Morphology and size distribution of dispersed nanoparticles were evaluated by TEM analysis (Fig. 5A). The obtained AuNPs

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**Fig. 1** Optimisation of AuNPs and thiol-modified oligonucleotide probes concentrations by gradually adding NaCl. 0.1 M (A1, B1, 12 h), 0.2 M (A2, B2, 24 h), and 0.4 M (A3, B3, 48 h) of NaCl
were spherical and diameters of particles were measured in the range of 3–9 nm with a mean diameter of 5.1 nm ($n = 50$).

To discriminate HPV types 16, 18, 31, 33, 45, 52, and 58, five sets of oligonucleotide probes (35–39 nucleotides in length, designed to achieve a $T_m$ for the binding of each probe to its complementary sequence of $\sim 45^\circ C$) were created to target the variable L1 region of the HPV genome. One common probe was designed for the purification of HPV DNA types 18, 31, and 33, and the other four sets were designed for the purification of HPV DNA types 16, 45, 52, and 58. The sequences of all probes used are shown in Table 1.

Conjugation between AuNPs and the oligonucleotide probes resulted in clear supernatant with dark red oily precipitate. The red oily precipitate was dispersed in 0.05 M NaCl solution. This maximum loading was obtained at an AuNP concentration of 1.06 µg/ml and a thiol-modified oligonucleotide concentration of 20 µM, performed in 20 µl volume. Minimal aggregation of Oligo-AuNP particles was observed, with an average size increase to 12.6 nm diameter and a range of 6–22 nm ($n = 100$, Fig. 5B). The efficiency of thiol-oligo binding to the particles was determined using the following equation:

$$\text{Efficiency} = \frac{A_0 - A_1}{A_0} \times 100$$  

In which $A_0$ is the absorbance of the oligonucleotide probe [optical density at 260 nm (OD 260 nm) $= 0.746$] and $A_1$ is the absorbance of the first obtained supernatant (OD 260 nm $= 0.030$). UV–Vis detection of unbound oligonucleotide in the supernatant showed nearly 95% of oligonucleotide probes to be attached to the AuNPs (Fig. 6). The zeta potential of Oligo-AuNPs was found to be $-137.7$ mV compared to $-67$ mV before modification. It should be noted that other nanoparticle sizes have not been tested and may not perform similarly [33].

Fig. 2  Schematic diagram shows DNA purification based on AuNPs by liquid phase hybridisation approach. Step 1: cell disruption using lysis buffer and proteinase K; step 2: denaturation of target DNA; step 3: hybridisation of AuNP probes to target DNA; step 4: detachment of AuNPs from the target HPV DNA

Fig. 3  UV–Vis spectra of AuNP and AuNP probes
3.2 Optimisation of hybridisation conditions

Hybridisation for 30 min at a temperature of 45°C was found to be optimal for all probes after a series of tests at differing temperatures and annealing times (Fig. 7), allowing the assay to be performed in a single tube containing a mixture of five sets of oligonucleotides. Under these optimised conditions, a real-time PCR assay targeting a 145 bp of HPV L1 gene was able to detect 504 out of 800 copies/µl of purified HPV DNA with an efficiency of >90%.

Fig. 4 DLS of AuNPs

Fig. 5 Morphology and size distribution of dispersed nanoparticles
(a) TEM of AuNPs, (b) TEM of Oligo-AuNP

Fig. 6 Optical density measurement for the calculation of loaded AuNPs with oligonucleotide probes at a wavelength of 260 nm
3.3 Performance verification of DNA purification method

DNA extraction, purification, and PCR efficiency have to be evaluated for each type of specimen, as these operations can be influenced by several experimental factors [34]. To monitor the DNA purification method efficiency, quantitative real-time PCR using GP5+/GP6+ consensus primers was performed on a serial 10-fold dilution of HPV DNA genotypes 16, 18, 31, 33, 45, 52, and 58 ranging from 10\(^{10}\) copies to 1 copy per reaction. PCR efficiency was determined from the slope of the standard curve for each genotype in a separate experiment. qPCR efficiency for genotypes 16, 18, 45, 52, and 58 were 8\(\lambda\), 10, 12, 14, and 16\%, respectively, which are considered acceptable for qPCR. The \(R^2\) values for all genotypes were 0.99 and the lower detection limit was 10 copies of HPV DNA per reaction.

The current gold standard for the viral load is qPCR. However, various PCR-based quantitative methods such as qPCR-enzyme immunoassay [35], TaqMan probes [36], fluorescence resonance energy transfer (FRET) probes [37], AllGlo probes [38], and more recently digital droplet PCR have been used for the detection and quantification of HPV DNA extracted from tissue samples [39]. Since the accurate limit of detection and limits of quantification of a PCR method mainly depend on the DNA extraction step, it is difficult to compare the detection limit of our method with others. Generally, in terms of detection limit, droplet digital PCR assay is highly sensitive and has the theoretical detection limit of a single copy of target DNA.

3.4 Sensitivity evaluation of the DNA extraction methods

We compared our AuNP-based purification method with a standard Qiagen FFPE DNA kit, employing specimens with known HPV DNA concentrations. The AuNP probes gave rise to detection of 733 out of 800 copies (91.6%) of HPV-16 DNA, above the Qiagen kit sensitivity (36 of 800 copies, 4.5%, Fig. 8). The threshold cycle value of DNA detection was thereby also lower for target DNA obtained from AuNPs than by Qiagen FFPE DNA kit extraction. The mean cycle threshold (Ct) value between AuNPs and the Qiagen kit in one case (blue) was as high as 10-fold differences, shown in Fig. 8B. These differences are to be expected given the lack of specificity in extraction by commercial tissue kit, and the targeted sequence extraction made possible by the use of specific probes on the AuNPs.

The AuNP-probe method has the additional advantage of simpler workflow, requiring five steps to obtain purified HPV-DNA as a template for PCR amplification as opposed to 21 steps in the manufacturer's protocol for total DNA purification from FFPE tissue samples. Besides, the traditional DNA extraction method requires xylene pre-treatment for deparaffinisation, which is time-consuming and negatively affects the quality of extracted DNA. To overcome this problem, we heated paraffin sections at 120°C before proteinase K treatment, resulting in higher quality and quantity of DNA extract. Of course, these improvements are purchased at the cost of specificity for our method cannot isolate DNA from papillomavirus or any other source that does not match the probe sequences.

Although different methods of HPV DNA detection based on nanoparticle have been introduced, the technique of DNA extraction used in the studies was based on the extraction of total DNA [40, 41]. However, this will affect the sensitivity of detection, even if it does not have much effect on the specificity of the test.
With the enzyme [42, 43]. To eliminate this problem, BSA in the tumour, and four randomly selected FFPE tissue samples positive for low-risk HPV genotypes 6, 53, 87, and λ0 previously tested with Ampliquality assay as a reference method were examined.

Table 2  Specificity of AuNPs-probe purification method using FFPE cervical cancer tissue samples

| No. | Genotype | Threshold cycle | DNA copies/reaction |
|-----|----------|-----------------|--------------------|
| 1   | HPV –16  | 33.27           | 2.14 × 10^2        |
| 2   | HPV-16   | 26.67           | 1.42 × 10^4        |
| 3   | HPV-16   | 34.93           | 7.49 × 10           |
| 4   | HPV-16   | 35.23           | 6.20 × 10           |
| 5   | HPV-16   | 33.69           | 1.65 × 10           |
| 6   | HPV-18   | 23.22           | 1.27 × 10^5        |
| 7   | HPV-33   | 36.63           | 2.55 × 10           |
| 8   | HPV-45   | 32.76           | 2.97 × 10^2        |
| 9   | HPV-52   | 31.19           | 8.06 × 10^2        |

3.5 Assessment of HR-HPV DNA purification in patient-derived samples

To evaluate the performance of our method on FFPE cervical specimens, we compared FFPE HPV genotyping results with the commercially available Ampliquality HPV-TYPE genotyping assay, which is based on single-step PCR and reverse line blot. Extracted DNA from nine FFPE cervical cancer tissue samples were tested for the presence of HR-HPV genotypes by two methods. AuNPs-Probe was able to isolate different HR-HPV genotypes specifically (Table 2). There was 100% concordance when the genotypes were tested by Ampliquality HPV-Type genotyping assay, indicating the excellent performance of the AuNPs-Probe HPV DNA genotype isolation method for FFPE specimens.

Also, nine HPV negative FFPE samples from benign ovarian tumour, and four randomly selected FFPE tissue samples positive for low-risk HPV genotypes 6, 53, 87, and 90 previously tested with Ampliquality assay as a reference method were examined. Real-time PCR assay yielded negative results, indicating a 100% specificity of the purification method. No false positive was found in negative controls by the newly designed AuNPs method. Samples showed no cross-reactivity with the designed specific probes as well.

One drawback of using AuNPs in the PCR assay is its negative effect on DNA polymerase activity, probably due to its bondage with the enzyme [42, 43]. To eliminate this problem, BSA in the concentration of 700 µg/ml and one unit of Taq DNA polymerase were added to the PCR master mix. Furthermore, the elution step was included in the procedure to separate the AuNP-Probe complex from the target DNA sequence to improve PCR efficiency [44].

4 Conclusion

A high yield DNA isolation method based on AuNP and probe hybridisation assay was introduced to improve the purification and detection of HR-HPV DNA in the FFPE tissue of cervical cancer. The method was formed based on AuNPs coated with common or specific oligonucleotide probes complementary to the HR-HPV L1 gene. The main strength of this method is the increased amount of purified DNA in comparison with the widely used QIAamp DNA FFPE Tissue Kit (91.6% versus 4.5%). Besides, the method reduced the number of steps in the procedure with a positive effect on the DNA yield. The developed method can be designed to purify various DNA from different clinical samples specifically. This type of specific extraction method can improve an accurate and rapid detection of HR-HPV DNA in FFPE samples for the clinical management of patients. Also, the method can be further improved for the purification of HPV DNA in different clinical samples. However, more samples have to be tested to evaluate their application in practice.

5 Acknowledgments

This work was based on a thesis in Medical Biotechnology by Noorossadat Seyyedi (Project No. 94–01–10–9150), supported by Shiraz University of Medical Sciences, Shiraz, Iran. The authors wish to thank Prof. M.G. Finn at the Georgia Institute of Technology and Mr A. Zare-Hoseinabadi for their scientific editing of the manuscript. We are also thankful to Mr H. Argasi at the Research Consultation Center (RCC) of Shiraz University of Medical Sciences for his invaluable assistance in editing this manuscript.

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