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We use this protocol and it's working

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# Protocol for Papillomavirus DNA extraction from cervical brushes using the Wizard® Genomic DNA Purification Kit

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ABSTRACT

DNA analysis for early diagnosis of cervical cancer is a promising alternative for greater treatment efficiency. Biological samples obtained from the endocervix are commonly used for diagnosis, even though such samples are still little explored in the molecular context. This work aims to describe a modified protocol for extracting HPV DNA from infected endocervical cells after collection with endocervical brushes. The Wizard® Genomic DNA Purification Kit (Promega, CAT.# A1125) was used to extract DNA from endocervical samples and the extraction protocol was adapted from the manufacturer's instructions. Forty-one samples were collected from women with a mean age of 35 ± 7.8 years. It was observed that samples containing higher blood content resulted in inhibiting the amplification signal, however, all samples had enough material for DNA extraction, despite the blood contamination. The quality of the extraction was confirmed in 100% of the samples with the amplification of the actin gene, indicating that enough DNA was extracted. Considering the absorbance ratio of 260/280, the average purification of the samples was reasonably good. The protocol was able to extract and detect HPV DNA with the potential to be used in the detection of high-risk HPV from cervical brushes with a high cost-effectiveness ratio.

## COLLECTION OF SAMPLES

1 For the collection of endocervical material, the speculum is inserted into the vaginal canal

2 After viewing the cervix, the brush is introduced into the cervical canal

3 Then the brush is rotated 360 degrees by eight turns to the right and eight to the left in order

4 After collection, the brush is transferred to a clean collection tube and stored at -80 °C

## DNA EXTRACTION

5 Add 600µl of Nuclei Lysis Solution to a 1.5 ml microcentrifuge tube free of DNAses, RNAses and pyrogens

6 Add the endocervical brush to the tube containing the Nuclei Lysis Solution and homogenize the sample by rotating the brush in the solution

7 Remove the brush and add 3µl of RNase Solution and mix the sample by inverting the tube 5 times

8 Incubate the mixture for 30 minutes at 37°C. Allow the sample to cool to room temperature for 5 minutes before proceeding

9 To the room temperature sample, add 200µl of Protein Precipitation Solution and vortex vigorously at high speed for 20 seconds. Chill sample on ice for 5 minutes

10 Centrifuge at room temperature at 14,000 x g for 4 minutes

11 Transfer supernatant to a fresh tube containing 600µl of room temperature 99.5% isopropanol

12 Mix gently by inversion and centrifuge for 1 minute at 14,000 × g at room temperature

13 Decant the supernatant and add 600µl of room temperature 70% ethanol, rinsing the tube walls and homogenizing by inversion

14 Centrifuge for 1 minute at 14,000 × g at room temperature and aspirate the ethanol

15 Invert the tube on clean absorbent paper, and air-dry the pellet for 15 minutes

16 Rehydrate the DNA in 100µl of DNA Rehydration Solution for 1 hour at 65°C. Periodically mix the solution by gently tapping the tube

17 Store the DNA at - 20°C