Supplementary Material

- Amplification of human papillomavirus (HPV) DNA
  Polymerase chain reaction (PCR) reaction mixture was prepared in the volume of 50 µL. The constituents included:
  - 5 µL DNA template,
  - 1 µL deoxynucleoside triphosphate (dNTPs) (10 mM),
  - 0.3 µL Taq DNA polymerase (5 U/µL),
  - 5µL 10X PCR buffer,
  - 1 µL of each GP5+ and GP6+ primers (30 pmol)
  - 36.7 µL DNase free water.

  For one sample the volume was taken for two reactions: one for sample and other for negative control. For negative control, 5 µL distilled water was taken.

Cycling conditions

- Initial denaturation, at 95°C for 5 minutes.
- 35 cycles of denaturation Tm, 95°C for 40 seconds, annealing Tm, 40°C for 40 seconds, and Extension Tm, 72°C for 45 seconds.
- The final extension cycle was 72°C for 3 minutes.
- Holding at 4°C.

- Procedure for one-step RT-PCR for reverse transcription and amplification for HPV E6/E7 mRNA detection
  - Template RNA, primer solutions, dNTP mix, 5x QIAGEN one step RT PCR buffer, 5X Q solution, and RNase-free water were thawed, placed on ice, and mixed thoroughly.
  - The primers were reconstituted in distilled water 10 times, i.e., 90 µL distilled water and 10 µL primers.
  - PCR reaction mixture was prepared in the volume of 50 µL according to the following table:

Component	Volume/ Reaction
• RNase free water	9 µL
• QIAGEN One step RT-PCR 5X buffer	10 µL
• dNTP's	2 µL
• 5X Q solution	10 µL
• Primers	
16/18/31/45 E6 FP	3 µL
16/18/31/45 E6 RP	3 µL
16/18/31/45 E7 FP	3 µL
16/18/31/45 E7 RP	3 µL
• QIAGEN One step RT-PCR Enzyme mix	2 µL
• Template RNA	5 µL

For one sample the volume was taken for two reactions: one for sample and other for negative control. For negative control, 5 µL distilled water was taken.

The reaction mixture was gently mixed and appropriate volumes were dispensed in PCR tubes.

The thermal cycler was run according to the following cycling conditions:

Cycling Conditions for ONE-STEP RT-PCR

Step	Time	Temperature
Reverse transcription	30 min	50°C
Initial PCR activation	15 min	95°C
Three step cycling		
Denaturation	0.5–1/21 min	94°C
Annealing	0.5–1 min	50–68°C
Extension	1 min	72°C
Number of cycles	25–40	
Final Extension	10 min	72°C

