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# A new approach for detecting HPV DNA in cervical swabs: comparison of nucleic acid extraction with direct PCR

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## Abstract

**Background** Almost all cases of cervical cancer are associated with persistent high-risk HPV infection. WHO prioritizes primary HPV testing for cervical cancer screening. Cervical cancer screening programs require the ability to process a large number of samples in a simple and standardized manner and obtain reliable results. The workload, time, and cost associated with the number of samples to be processed encourage the development of alternative methods to the traditional nucleic acid extraction method for population-based screening. In this study, we aimed to compare the performance of a commercial pre-denaturation solution with nucleic acid extraction in HPV DNA detection by PCR in cervical swab samples.

**Methods** The study was designed in two phases: an experimental phase and a clinical phase. A total of 1200 cervical swabs were included in the clinical phase of the study. Positive results were obtained in 143 (11.9%) samples by nucleic acid extraction and 137 (11.4%) samples by PharmaDirect. Discordant results were detected in 28 (2.3%) samples.

**Results** PharmaDirect provided 88.1% sensitivity compared to nucleic acid extraction. PharmaDirect provided high sensitivity for HPV genotype 16 (92.3%) and relatively limited sensitivity for mixed genotype infections (73.7%).

**Conclusion** This study demonstrates the potential of an alternative commercial pre-denaturation product that does not require nucleic acid extraction for HPV DNA detection in cervical swab samples. Such approaches may represent a useful alternative for population-based screening studies.

**Trial registration** Not applicable.

**Keywords** HPV, HPV DNA, PCR, Pre-denaturation, Sensitivity, Cervical cancer screening

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## Introduction

Infection with one or more high-risk strains of human papillomavirus (HPV) can result in the development of cervical intraepithelial neoplasia. Among women, cervical cancer is the fourth leading cause of cancer-related death globally, with certain HPV types posing a risk for cancer progression [1]. Currently, more than 200 HPV genotypes are known to infect humans [2]. The World Health Organization (WHO) reported that 14 different HPV genotypes, which are 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68, as high-risk cancer-causing HPV genotypes in 2024 [1]. According to the World Health Organization (WHO, 2024), HPV is responsible for more than 95% of the estimated 660,000 new cervical cancer cases diagnosed globally each year. Over 90% of the estimated 348,000 cervical cancer deaths in 2022 occurred in low- and middle-income countries [1, 3]. The International Agency for Research on Cancer (IARC) reported on August 1, 2024, that 17 HPV genotypes are associated with cervical cancer. HPV types 16 and 18 are the most prevalent, causing nearly 75% of cases worldwide, while types 31, 33, 45, 52, and 58 contribute an additional 15–20%, underscoring their significant role in global cervical cancer incidence [4]. Additionally, high-risk HPV infection can contribute to abnormal pregnancy outcomes, including cervical infections, premature birth, and placental disorders [5]. Vaccination has become the primary prevention strategy against HPV infections due to technological advances over the last 25 years. Incorporating vaccination into cervical cancer screening programs represents an effective strategy for preventing the onset of cervical cancer [6]. Screening methods for cervical cancer include the cytology-based Papanicolaou (Pap) test, the HPV-DNA test, and co-testing, which combines both cytological and molecular techniques [7]. For cervical cancer detection, HPV-DNA testing is known for its high sensitivity in identifying clinically significant lesions. Agorastos et al. (2022) found that incorporating HPV-DNA testing, compared to cytology, resulted in 188% increase in colposcopy referrals and 86% increase in case detection [8]. Another study concluded that a negative HPV-DNA test result is more reliable than a negative cytology result [9]. Although HPV-DNA-based screening is highly effective in detecting high-risk HPV infections, many HPV-positive women, particularly those in the 20–45 age range, may remain asymptomatic. As a result, triage strategies are essential to identify those who require further diagnostic evaluation or treatment. HPV-DNA screening has demonstrated high sensitivity, negative predictive value (NPV), and positive predictive value (PPV), making it particularly effective in this age group for identifying high-risk cases [10].

Directed screening for oncogenic HPV types in targeted populations may offer a more effective approach for triaging individuals who require further diagnostic evaluation or therapeutic intervention. Moreover, precise molecular diagnostic techniques are essential for assessing the long-term efficacy of HPV vaccination programs, providing critical insights into the persistence of high-risk HPV infections and the reduction of HPV-related lesions over time [11]. E1 or L1 open reading frames are commonly targeted in PCR-based HPV-DNA tests used for cervical screening [12]. Real-time PCR (7qPCR), offering rapid results within a few hours and being both cost-effective and partially automated, represents a practical approach for large-scale population-based screening initiatives.

The population-based cervical cancer screening program in Turkey uses the HPV-DNA test method [13]. HPV-DNA tests require relatively small DNA quantities, but population-based studies necessitate the capability to process multiple samples in a standardized and straightforward manner while ensuring reliable results [14]. The increasing workload, time demands, and costs associated with processing large numbers of samples have led to the development of alternative methods to traditional nucleic acid extraction techniques in population-based screening [15, 16]. Although urine samples offer promising potential for HPV-DNA detection, advancements are needed to improve the efficiency and reliability of electrochemical DNA chips for this purpose. Additionally, to fully develop diagnostic tools for gynecological cancers, challenges such as cost, fabrication time, complexity, and data interpretation must be addressed [17].

The PharmaDirect, developed by Pharmaline Health Services Industry and Trade Inc., is a pre-denaturation solution designed to facilitate direct PCR analysis of cervical swab samples, without the need for nucleic acid extraction. This study presents a comparative analysis of the PharmaDirect solution and a commercially available magnetic bead-based nucleic acid extraction kit for PCR-based HPV DNA detection in cervical swab samples.

## Materials and methods

This study was conducted in June 2024 at the National HPV Laboratory of the Republic of Türkiye Ministry of Health, General Directorate of Public Health, Microbiology Reference Laboratories and Biological Products Department. The study was planned as two phases, experimental and clinical.

### Experimental phase

In the experimental phase of the study, 80 cervical swab samples collected from sexually active women aged 30–65 years were utilized. These samples had already undergone routine nucleic acid isolation and PCR testing in the laboratory, with confirmed positive or negative results.

The samples analyzed in this study were cervical swabs, from which HPV status was determined using PCR. Colposcopy and biopsy were not included as part of the diagnostic protocol for these samples.

After routine testing, the samples were separated, labeled in 2 mL vials of Viral Transport Medium DiaVTM, (SBT, Türkiye), and transported to the laboratory for experimental comparison of methodologies. They were then stored at -80 °C for further use in the study described in this article. The test results obtained using the Molgen PurePrep Pathogen DGX (Molgen, The Netherlands) nucleic acid extraction and multiplex HPV DNA PCR test were compared with the results of the PharmaDirect methodology, as outlined in the study design (Fig. 1).

### Clinical phase, sample collection and processing

The clinical phase of the study included 1200 sexually active women aged 30–65 years. Cervical swabs were collected from each participant, and the samples were placed in 2 mL of Viral Transport Medium, DiaVTM (SBT, Türkiye). These samples were then transported to the laboratory for use in this study.

### Nucleic acid extraction using molgen pureprep pathogen DGX kit

Extraction steps were performed on an automated PurePrep96 (Molgen, The Netherlands) extraction device according to the manufacturer's recommendations. Extraction of 80 samples as part of routine laboratory work and 1200 samples as part of clinical work was performed in 96-well plates. 50 µl of nucleic acid was extracted from each sample.

### Real-time HPV PCR testing with nucleic acid extraction

Extracts were tested with TÜSEB DiaKit HighRisk HPV qPCR diagnostic kit (SBT, Türkiye). TÜSEB DiaKit was developed for the detection of HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 nucleic acids in cervical swab samples. Using this kit, HPV genotypes 16, 18 and 45 are detected in separate fluorescent channels, while other genotypes are tested in a single fluorescent channel.

In accordance with the manufacturer's recommendations, the main reaction mixture for nucleic acid extracts was prepared as follows; 5 µl Mastermix – 2.5 µl Primer Mix – 2.5 µl nucleic acid extract. The prepared reaction mixture was tested with the CFX96 Touch Real-Time PCR Detection System (Bio-Rad, USA). The cycling conditions used

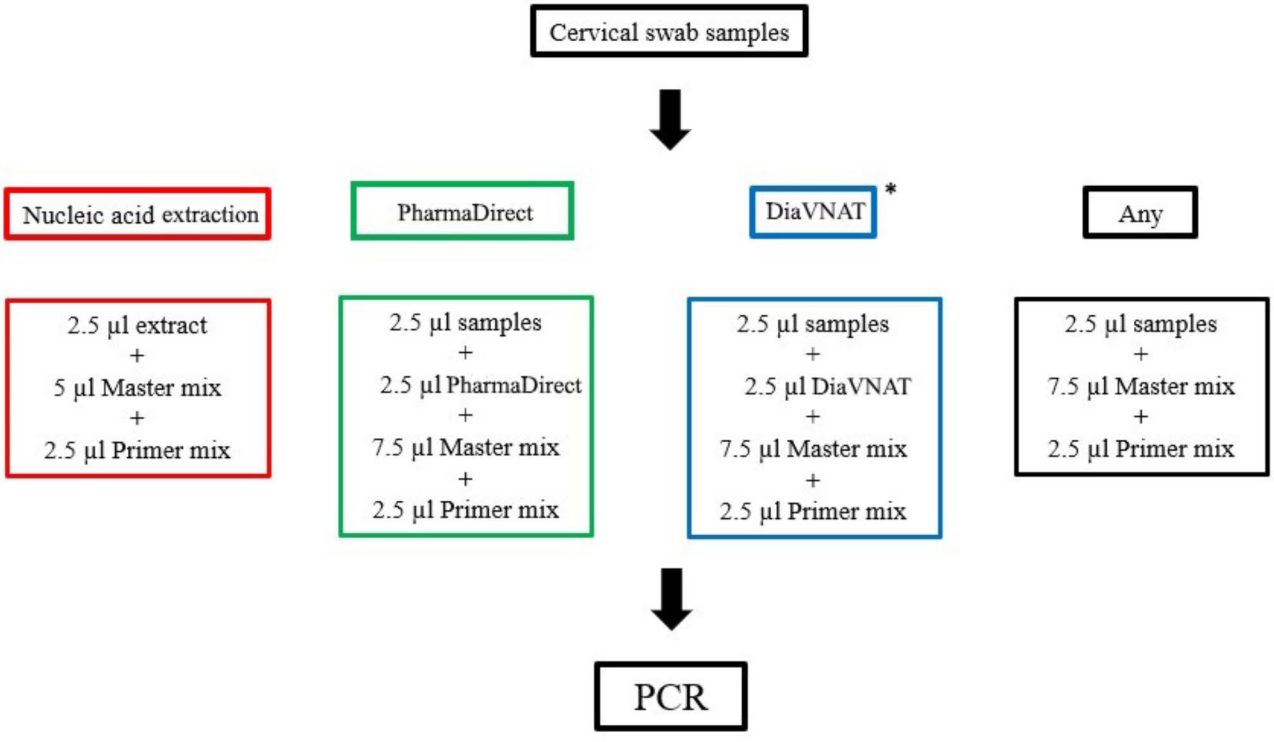


**Fig. 1** Flow chart of different HPV-DNA detection methods compared in the experimental phase of the study. Nucleic Acid Extraction; DNA purification was performed from cervical swab samples. Purified DNA was included in the PCR reaction. PharmaDirect; was added in the PCR reaction along with the cervical swab sample without nucleic acid extraction. DiaVNAT*; A Viral nucleic acid buffer (SBT, Türkiye) used in respiratory tract swabs, was added in the PCR reaction along with the cervical swab sample without nucleic acid extraction. Any; Only the cervical swab sample was included in the PCR reaction, with no additional solutions added

**Table 1** Test results obtained with the PCR in the experimental phase of the study

| hrHPV genotypes | NAI | PharmaDirect | DiaVNAT | Any |
|---|---|---|---|---|
| Positive | **53 (66.3)** | **44 (55.0)** | **35 (43.8)** | **31 (38.7)** |
| HPV genotype 16 | 19 (23.8) | 17 (21.2) | 11 (13.8) | 10 (12.5) |
| HPV genotype 18 | 7 (8.8) | 7 (8.8) | 6 (7.5) | 3 (3.7) |
| HPV genotype 45 | 4 (5.0) | 4 (5.0) | 4 (5.0) | 4 (5.0) |
| Other genotypes | 23 (28.7) | 16 (20.0) | 14 (17.5) | 14 (17.5) |
| Negative | 27 (33.7) | 36 (45.0) | 45 (56.2) | 49 (61.3) |
| Total | **80 (100.0)** | **80 (100.0)** | **80 (100.0)** | **80 (100.0)** |

hrHPV: high-risk human papillomavirus, NAI: nucleic acid extraction, Total: Number of identical samples included in the study for each method: Ratio of the number of samples in each category to the total number of samples in the method (percentage)

for PCR assays consisted of 40 cycles of 95 °C for 3 min, followed by 95 °C for 5 s and 60 °C for 10 s, in line with the manufacturer's recommendations. Negative and positive controls were included in each study.

### Real-time HPV PCR testing with Pharmadirect pre-denaturation solution

Samples were tested with TÜSEB DiaKit HighRisk HPV qPCR diagnostic kit. In accordance with the manufacturer's recommendations, the main reaction mixture used for nucleic samples was prepared as follows; 7.5 µl Mastermix – 2.5 µl Primer Mix – 2.5 µl PharmaDirect – 2.5 µl sample. The prepared reaction mixture was tested using the CFX96 Touch Real-Time PCR Detection System. The PCR cycling conditions included 35 cycles of 95 °C for 3 min, followed by 95 °C for 10 s and 60 °C for 45 s, in accordance with the manufacturer's recommendations. Both negative and positive controls were included in each assay. For experimental comparison, DiaVNAT was used instead of PharmaDirect in the reaction under the same conditions. As an additional comparison methodology, the study was conducted without adding any solution to the reaction, using the same conditions.

### Statistical analysis

In the clinical phase of the study, HPV DNA PCR test results obtained from the two methodologies were combined and presented in a cross-tabulation. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for PharmaDirect solution were calculated by creating a comparative table, considering nucleic acid extraction as the gold standard. The agreement between the test results obtained with both methodologies was analyzed by calculating Cohen's Kappa values were categorized as follows: below 0.20 indicates insignificant agreement, 0.21–0.40 indicates poor agreement, 0.41–0.60 indicates fair agreement, 0.61–0.80 indicates substantial agreement, and 0.81–1.00 indicates great agreement [18]. Ct mean values obtained in PCR tests performed using both methodologies for HPV genotypes 16, 18, 45 and other high-risk genotypes were compared with the help of Paired Sample T-test.

**Table 2** PCR assay results obtained after nucleic acid extraction and pre-denaturation with PharmaDirect [n (%)]

| PharmaDirect | Nucleic acid extraction | | |
|---|---|---|---|
| | Positive | Negative | Total |
| Positive | 126 (10.5) | 11 (0.9) | 137 (11.4) |
| Negative | 17 (1.4) | 1046 (87.2) | 1063 (88.6) |
| Total | 143 (11.9) | 1057 (88.1) | 1200 (100.0) |

## Results

During the experimental phase of the study, 80 cervical swabs were processed using the four methodologies outlined above. At this stage, 53 (66.3%) samples were positive by nucleic acid extraction, 44 (55.0%) by PharmaDirect, 35 (43.8%) by DiaVNAT and 31 (38.7%) by the last methodology (Table 1).

In light of the experimental phase data, the performance of the PharmaDirect solution was compared with nucleic acid extraction during the clinical phase of the study. For this purpose, a total of 1200 cervical swab samples were processed using both the nucleic acid extraction and PharmaDirect methods and tested by PCR. Positive results were obtained in 143 (11.9%) samples by nucleic acid extraction and 137 (11.4%) samples by PharmaDirect. Negative results were obtained with PharmaDirect in 17 (1.4%) samples that were positive by nucleic acid extraction. 11 (0.9%) samples that were negative by nucleic acid extraction were found

**Table 3** Distribution of nucleic acid extraction and PCR positive test results by PharmaDirect methodology

| PCR Test Result | Extraction | | PharmaDirect | |
|---|---|---|---|---|
| | *n* | % | *n* | % |
| **HPV genotype 16** | 40 | 28.0 | 40 | 29.2 |
| **HPV genotype 18** | 12 | 8.4 | 13 | 9.5 |
| **HPV genotype 45** | 10 | 7.0 | 11 | 8.0 |
| **Other** | 62 | 43.3 | 57 | 41.6 |
| **Mixed positive** | 19 | 13.3 | 16 | 11.7 |
| 16 and 18 | 1 | 0.7 | 1 | 0.7 |
| 16 and 45 | 2 | 1.4 | 2 | 1.5 |
| 16 and Other | 7 | 4.9 | 9 | 6.6 |
| 18 and Other | 4 | 2.8 | 1 | 0.7 |
| 45 and Other | 3 | 2.1 | 2 | 1.5 |
| 16, 18 and Other | 2 | 1.4 | 1 | 0.7 |
| **Total** | **143** | **100.0** | **137** | **100.0** |

**Table 4** Sensitivity, specificity, PPV and NPV values for PharmaDirect solution, considering nucleic acid extraction as the gold standard

| | I+/PD+ (TP) | I+/PD- (FN) | I-/PD+ (FP) | I-/PD- (TN) | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) |
|---|---|---|---|---|---|---|---|---|
| HPV genotype 16 | 48 | 4 | 5 | 1143 | 92.3 | 99.6 | 90.6 | 99.7 |
| HPV genotype 18 | 15 | 4 | 1 | 1180 | 78.9 | 99.9 | 93.8 | 99.7 |
| HPV genotype 45 | 15 | - | - | 1185 | 100 | 100 | 100 | 100 |
| Other genotypes | 63 | 15 | 7 | 1115 | 80.8 | 99.4 | 90 | 98.7 |
| Mixed genotypes | 14 | 5 | 2 | 1179 | 73.7 | 99.8 | 87.5 | 99.6 |
| **Total** | **126** | **17** | **11** | **1046** | **88.1** | **99.0** | **92.0** | **98.4** |

I+: Number of samples with positive results by nucleic acid extraction, I-: Number of samples with negative results by nucleic acid extraction, PD+: Number of samples with positive results by PharmaDirect methodology, PD-: Number of samples with negative results by PharmaDirect methodology, TP: True positive, FN: False negative, FP: False Positive, TN: True negative, PPV: Positive predictive value, NPV: Negative predictive value

positive using PharmaDirect (Table 2). The genotype distribution of the samples that were PCR positive by nucleic acid extraction and PharmaDirect methodology is given in Table 3.

When nucleic acid extraction was considered the gold standard, the sensitivity, specificity, PPV and NPV for the PharmaDirect solution were 88.1%, 99%, 92% and 98.4%, respectively. The sensitivity, specificity, PPV and NPV values determined for the PharmaDirect solution for each different HPV genotype are presented in detail in Table 4.

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The Kappa values indicating the level of agreement between the test results obtained from both methodologies are presented in Table 5. A "great agreement" was observed between the qualitative results of both methodologies (Kappa = 0.868, $p < 0.001$).

Ct mean values obtained with both methodologies in PCR positive samples are given in Table 6. For HPV genotype 18 positive samples, similar Ct mean values were obtained with both methodologies (p:0.303). On the other hand, different Ct mean values were determined for HPV genotypes 16, 45 and other genotypes with both methodologies ($p < 0.05$).

**Table 5** Kappa values showing the agreement between both methodologies

| HPV genotypes | Nucleic acid extraction | | PharmaDirect | | κ (95% CI) |
|---|---|---|---|---|---|
| | *n* | % | *n* | % | |
| HPV genotype 16 | 52 | 4.3 | 53 | 4.4 | **0.91** (0.88–0.94) |
| HPV genotype 18 | 19 | 1.6 | 16 | 1.3 | **0.855** (0.791–0.919) |
| HPV genotype 45 | 15 | 1.3 | 15 | 1.3 | **1.00** (1.00–1.00) |
| Other | 78 | 6.5 | 70 | 5.8 | **0.842** (0.809–0.875) |
| **Total*** | **143** | **11.9** | **137** | **11.4** | **0.868** (0.846–0.890) |

** Total number of samples with positive results obtained by the test technique*

## Discussion

Screening programs necessitate the ability to process samples in a straightforward and standardized manner while ensuring reliable results. Given the high volume of samples, along with considerations of time and cost, it may be prudent to develop alternative methods to the traditional nucleic acid extraction technique for use in population-based screening studies [14–16]. PharmaDirect is a commercial pre-denaturation product developed to enable direct PCR analysis of cervical swab samples without nucleic acid extraction.

Humanity has recently faced the COVID-19 pandemic, which has impacted the entire world [19]. The PCR technique has become an essential tool for microbiology laboratories in managing the diagnostic burden associated with the pandemic [20]. During this period, researchers sought to develop alternative methods for detecting SARS-CoV-2 RNA in respiratory samples by PCR analysis that did not require nucleic acid extraction [21–25].

To the best of our knowledge, this is the first study to investigate an alternative to nucleic acid extraction for HPV DNA detection by PCR in cervical swab samples. In the experimental phase, which was conducted in two distinct phases, a higher level of agreement was observed between the gold standard nucleic acid extraction and the PharmaDirect method compared to the other two alternative methodologies (Table 1).

HPV DNA was found in 143 (11.9%) samples that underwent nucleic acid extraction at the clinical stage and 137 (11.4%) samples that underwent PharmaDirect (Table 2). There was " great agreement" between PharmaDirect and nucleic acid extraction (Kappa = 0.842, $p < 0.001$) (Table 5). For PharmaDirect, sensitivity was 88.1%, specificity 99%, PPV 92% and NPV 98.4% compared to the gold standard method (Table 4).

Mahmoud et al. reported sensitivity 85.3%, specificity 95%, PPV 91.6%, NPV 91.3% and Kappa = 0.797 in PCR tests performed with an alternative method that does not require nucleic acid extraction for molecular diagnosis of SARS-CoV-2 [26]. Other researchers have also reported that alternative methods provide acceptable levels of sensitivity against extraction-dependent protocols [25, 27].

**Table 6** Paired sample T-test analysis for PCR positive samples with both methodologies

| Variables (Ct values) | | *N* | Mean | SD | Mean Difference | T | df | *p* |
|---|---|---|---|---|---|---|---|---|
| HPV genotype 16 | NAI | 48 | 24.14 | 3.18 | 1.86 | 4.04 | 47 | 0.000 |
| | PD | 48 | 26.00 | | | | | |
| HPV genotype 18 | NAI | 15 | 25.53 | 3.71 | 1.03 | 1.07 | 14 | 0.303 |
| | PD | 15 | 26.56 | | | | | |
| HPV genotype 45 | NAI | 15 | 25.41 | 3.07 | 3.40 | 4.29 | 14 | 0.001 |
| | PD | 15 | 28.81 | | | | | |
| Other genotypes | NAI | 63 | 23.80 | 2.57 | 2.59 | 7.97 | 62 | 0.000 |
| | PD | 63 | 26.39 | | | | | |

NAI: PCR test result after nucleic acid extraction, PD: PCR test result after PharmaDirect procedure, SD: Standard Deviation

The sensitivity and specificity levels obtained in this study were consistent with those reported in the literature. PharmaDirect demonstrated very high sensitivity for HPV genotypes 45 (100%) and 16 (92.3%). However, this methodology exhibited relatively lower sensitivity, particularly for HPV genotype 18 and mixed genotype infections. (Table 4).

In our study, higher Ct mean values were obtained with PharmaDirect in PCR positive samples (p:0.303 for HPV genotype 18 and $p < 0.05$ for HPV genotypes 16, 45 and other genotypes). Similarly, higher Ct mean values were obtained in SARS-CoV-2 PCR studies performed with alternative methods that did not require nucleic acid extraction compared to nucleic acid extraction in the study by Kang et al. [28].

The researchers noted that the nucleic acid extraction method used in their study resulted in a four-fold increase in concentration. In contrast, alternative methods that did not require extraction reduced the nucleic acid concentration by half, due to a 1:1 dilution of the sample. They also indicated that this dilution could lead to an eight-fold variation in amplification results when comparing the same samples [28]. Theoretically, in an ideal environment with 100% PCR efficiency, a 10-fold change in sample concentration corresponds to a difference in Ct value of 3.3 [28, 29]. The collection and transfer of cervical swab samples to the laboratory, the specific pH environment of the genital tract, and the presence of human-derived epithelial cells and other proteins are factors that can impact nucleic acid extraction and PCR efficiency. Additionally, our study observed differences in HPV DNA concentration between methodologies, consistent with the findings of Kang et al. We believe these factors contribute to the observed mean differences in Ct values for each HPV genotype in our study.

## Conclusion

This study demonstrates the potential of a commercial pre-denaturation product that does not require nucleic acid extraction for HPV DNA detection in cervical swab samples. We hypothesize that such approaches could offer a valuable alternative for population-based screening studies.

Many different high-risk HPV genotypes are known to confer risk for the development of cervical cancer. In this study, the efficacy of PharmaDirect was relatively limited in HPV genotype 18 and mixed genotype infections.

To thoroughly evaluate the potential of the PharmaDirect method for clinical applications, it is essential to conduct more extensive studies. These studies should include a diverse range of populations, various laboratories and devices, and operators with differing levels of experience to accurately assess the method's reliability in clinical settings. Integration studies with different nucleic acid extraction kits and PCR systems could enhance the method's flexibility, allowing its use under varying laboratory conditions.

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**Author contributions**
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**Data availability**
No datasets were generated or analysed during the current study.

## Declarations

**Ethics approval and consent to participate**
Before starting the study, written permission was obtained from the Republic of Türkiye Ministry of Health, General Directorate of Public Health. This study was also approved by the Scientific Research Evaluation and Ethics Committee of Ankara Etlik City Hospital. (date 05.06.2024 and number AEŞH-BADEK-2024-389).

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare no competing interests.

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