



## Liquid-Based Cytology and HPV Co-testing in Cervical Cancer Screening for Female Sex Workers: Efficacy and Implications

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### Declaration

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### ABSTRACT

**Introduction:** Cervical cancer is a significant global health concern related to persistent high-risk HPV infection. Female sex workers (FSWs) are particularly vulnerable. Despite improved screening and prevention, FSWs in developed countries have a disproportionate risk. Liquid-based cytology (LBC) and HPV co-testing are emerging as promising tools for screening, offering improved sensitivity and specificity compared to conventional cytology alone. This research article aims to address the efficacy of LBC and HPV co-testing in cervical cancer screening. **Materials and methods:** This cross-sectional study whereby female sex workers (FSWs) in Pakistan aged 20 years and above were screened for cervical cancer using human papillomavirus (HPV) genotyping and Liquid-based cytology (LBC). **Results:** We collected data from 50 female sex workers. On HPV DNA testing, 6(12%) of them tested positive for HPV DNA, 3(6%) of them had HPV 16 infection, 2(4%) had HPV 52, and 1(2%) had HPV 59 infection. The other 44(88%) tested negative for HPV DNA. On liquid-based cytology, 5(10%) showed LSIL, 1(2%) showed ASC-H, and 1(2%) showed ASC-US. Three of the females who were detected positive for HPV also showed changes in LBC. On comparing both techniques, HPV DNA testing showed 82.14% sensitivity and 84% diagnostic accuracy. However, the P-value was significant for both of the techniques. **Conclusion:** The conventional pap smear, historically the primary method for cervical cancer screening, has limitations, including inadequate sample collection and low sensitivity. Liquid-based cytology and HPV DNA testing are newer methods with benefits and limitations. Combining methods yields good sensitivity and reduces missed diagnoses, making them valuable screening tools.

### INTRODUCTION

Cervical cancer is a global health issue. It is caused by persistent infection of high-risk human papillomavirus (HPV)(1). Usually, the human body clears the infection naturally within a period of 1 year and hence there will be no progression to cervical cancer. However, in some cases, the virus persists and can lead to the development of precancerous lesions and, eventually, cancer. That's why early detection through regular screenings is so important.

Marginalized populations such as female sex workers (FSWs) are most vulnerable due to various socioeconomic and behavioral factors. Despite improved screening and prevention, FSWs face disproportionate cervical cancer risk in developed countries(2). In developing countries like Pakistan, where there is no screening program and limited access to preventive healthcare, the risk of cervical cancer is dangerously high. Keeping in view these facts, there is an urgent need for tailored and effective screening approaches to mitigate the risk of cervical cancer.

Cervical cancer screening has evolved significantly over the years. One of the latest techniques is liquid-based technology, replacing conventional smears. This method has been found to improve the quality of the sample and reduce the number of inadequate smears(3). HPV DNA testing is a relatively new technique. It is susceptible to detecting high-risk HPV strains. Unlike cytology-based methods, HPV DNA tests are a more direct way of identifying the presence of HPV infection at a very early stage(4).

Liquid-based cytology (LBC) and human papillomavirus (HPV) co-testing have been emerging as a promising tool in cervical cancer screening. It has been thought to provide improved sensitivity and specificity compared to conventional cytology alone(5). LBC and enhancing sample adequacy also detect a wide range of cervical abnormalities. However, HPV co-testing provides essential information regarding high-risk HPV infection, which is an important risk factor for the development of

cervical cancer (6). More research is needed on the implications of co-testing, specifically on FSWs. Further research is required to understand how co-testing can support this population's cervical health.

This research article aims to address the efficacy of LBC and HPV co-testing in cervical cancer screening and implicate these strategies on a broader scale. We hope to improve cervical cancer outcomes among FSWs by improving early detection and management(7).

## MATERIALS AND METHODS

This is a cross-sectional study whereby female sex workers (FSWs) in Pakistan aged 20 years and above were screened for cervical cancer using human papillomavirus (HPV) genotyping and Liquid-based cytology (LBC).

The study received Institutional Review Board (IRB) approval from the Chughtai Institute of Pathology. Participants signed an informed consent form, and the data collected complied with relevant patient data protection rules and guidelines.

The project ran from 26th Feb,2024 to 25th Feb,2025, a questionnaire collected demographic information, sexual behavior history, and other significant information. Cervical samples were collected using liquid-based cytology techniques.

All specimens are tested for HPV DNA using the Anatolia Geneworks Bosphore HPV high-risk detection kit v1 on the Montania 4896 instrument, using real-time PCR. All the samples are run with positive and negative controls.

The LBC samples are processed at our lab using the Hologic thin prep 5000 processor according to the manufacturer's recommendations. Sample processing follows standardized protocols to ensure accuracy and reliability. A certified histopathologist analyzes the slides. The LBC is reported according to the third edition of the Bethesda System for Reporting Cervical Cytology. Statistical Analyses are done using SPSS. Descriptive statistics that summarized participant characteristics and screening results.

## RESULTS

We collected data from 50 female sex workers. Of these, 15(30%) females were less than 30, and 35(70%) were more than 30years of age. On HPV DNA testing, 6(12%) of them tested positive for HPV DNA, 3(6%) of them had HPV 16 infection, 2(4%) had HPV 52, and 1(2%) had HPV 59 infection. The other 44(88%) tested negative for HPV DNA. On liquid-based cytology, 5(10%) showed LSIL, 1(2%) showed ASC-H, and 1(2%) showed ASC-US. Three of the females who were detected positive for HPV also showed changes in LBC. All of them had a clinical history of vaginal

discharge. The sensitivity, specificity, and positive and negative predictive values of HPV DNA testing and liquid-based cytology are described below.

**Table 1**

*Diagnostic accuracy of liquid-based cytology in screening of cervical cancer.*

	Positive result on clinical findings	Negative result on clinical findings	P-value
Positive result on liquid based cytology	20 (TP)*	03 (FP)***	0.0001
Negative result on liquid based cytology	04 (FN)**	23 (TN)****	
<b>X2</b>		25.89	

\*-TP=True positive \*\*-FP=False positive \*\*\*-FN=False negative \*\*\*\*-TN=True negative

**Sensitivity:** 83.33%

**Specificity:** 88.46%

**Positive Predictive Value (PPV):** 86.96%

**Negative Predictive Value (NPV):** 85.19%

**Diagnostic Accuracy:** 86.0%

**Table 2**

*Diagnostic accuracy of HPV DNA in screening of cervical cancer.*

	Positive result on clinical findings	Negative result on clinical findings	P-value
Positive result on VIA	23 (TP)*	03 (FP)***	0.0001
Negative result on VIA	05 (FN)**	19 (TN)****	
<b>X2</b>		23.16	

\*-TP=True positive \*\*-FP=False positive \*\*\*-FN=False negative \*\*\*\*-TN=True negative

**Sensitivity:** 82.14%

**Specificity:** 86.36%

**Positive Predictive Value (PPV):** 88.46%

**Negative Predictive Value (NPV):** 79.17%

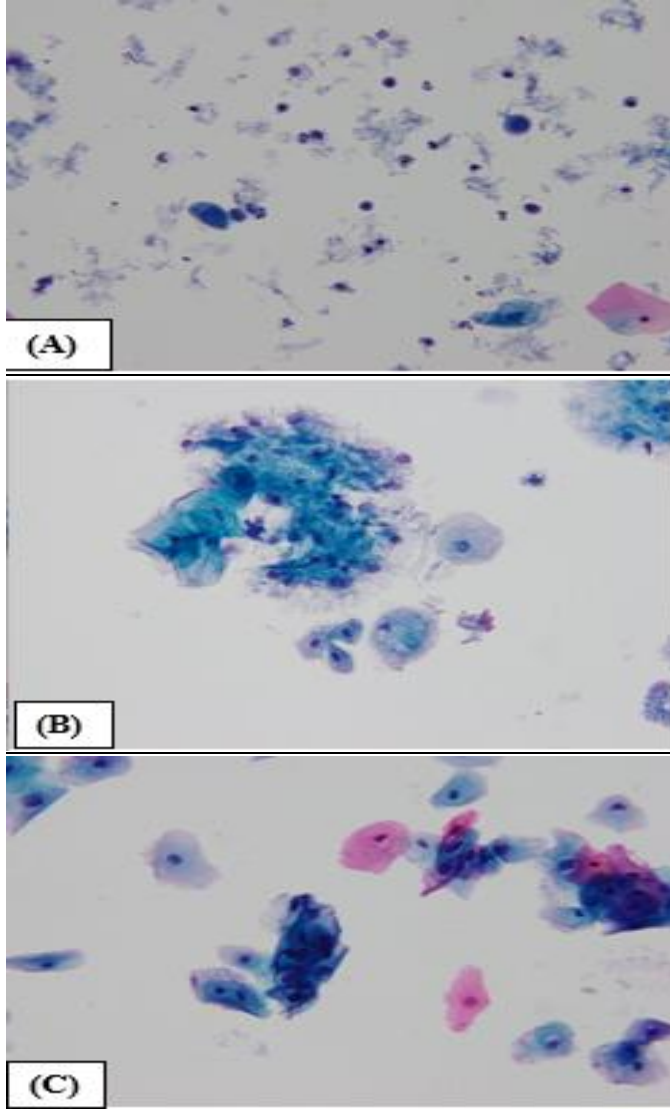
**Diagnostic Accuracy:** 84.0%

HPV DNA testing showed 82.14% sensitivity and 84% diagnostic accuracy, which was relatively less than cytology, which might be due to the limited number of our cases. The specificity of HPV was less than cytology, and the chances of misdiagnosis were increased, which might be due to transient infection of HPV at the same time. However, LBC and HPV DNA co-testing increased the sensitivity and diagnostic accuracy. A comparison of both techniques is given in Table 3. The P-value was significant for both of the techniques.

**Table 3**

*Comparison of both techniques*

RESULTS	SENSITIVITY	SPECIFICITY	PPV*	NPV*	X2	p-value
LIQUID BASED CYTOLOGY	83.33%	88.46%	86.96%	85.19%	25.89	0.0001
HPV DNA	82.14%	86.36%	88.46%	79.17%	23.16	0.0001

**FIGURE****(A) ASCUS(100x). (B) ASC-H(400x). (C) LSIL(400x).****DISCUSSION**

Cervical cancer is the fourth most common cancer in women globally(8), with around 660,000 new cases and around 350,000 deaths reported in 2022, according to WHO. Early detection and management of cervical cancer can lead to effective treatment. The higher incidence is in developing countries with low socio-economic status, lack of screening services, HPV vaccinations, and effective treatment strategies. There are many social and economic determinants, such as the prevalence of HIV, sex, STDs, smoking, and poverty, which lead to cervical cancer(9).

Human papilloma virus is the major risk factor of cervical cancer, with more than 200 types found. Many types of HPV associated infections remain asymptomatic while others can lead to viral warts or premalignant conditions (especially in cervix, vagina, anus, or oropharynx). HPV 16 and 18 account for almost 70% of all cases. Most of the spread is through sexual contact(9). However, early age of first sexual intercourse, multiple sexual partners, cigarette smoking and weak immunity can be the cause of HPV infections(10). Persistent HPV infection of almost 15-20 years can lead to development of

cancer from atypical cells, while women with weaker immune systems, such as due to HIV, are at risk of developing cancer in almost 5-10 years. Vaccination at 9-14 years of age is highly effective in preventing HPV infection. According to WHO, recommended age of screening is 30 years while 25 years in women with HIV infection. Screening is recommended for early detection and management of cervical cancer.

In the past, conventional pap smear was considered the most adopted method for cervical cancer screening. But it has limitations, such as inadequate sample collection, sample preparation errors, and the possibility of inaccurate microscopic examination. It has low sensitivity and specificity. It is replaced by liquid-based cytology, which has its benefits and limitations, such as morphological observation(10). There are latest updates in the cervical cancer screening guidelines which recommend the addition of Human papilloma virus DNA testing in the cervical cytological examination. Cervical specimens can be used for HPV DNA testing by nucleic acid amplification with polymerase chain reaction(PCR). American College of Obstetricians and Gynaecologists has recommended cervical cytology for every three years for women (aged 21 to 29). For women (aged 25 to 29), HPV testing alone can be considered, but cytology is preferred, while women (aged 30 to 65) cervical cytology and HPV DNA co-testing for every five years, cervical cytology alone for every three years, or HPV DNA testing alone for every five years is recommended(11) (12). The sensitivity of cytological results for high grade neoplasms can be increased from 50-85% to 100% on combining HPV DNA testing with liquid-based cytology(13). But HPV DNA testing is expensive, demands laboratory expertise and is time consuming, which is main issue in performing this test.

In our research, we collected data from 50 female sex workers, and few of them had a clinical history of vaginal discharge. Both LBC and HPV DNA testing were performed. There were 3 HPV 16 detected cases, while the other 3 had HPV 52 and HPV 59; the sensitivity and specificity were 82.14 & 86.36%, respectively. While the cytology of 5 females showed LSIL, the sensitivity and specificity were 83.33% & 88.46%, respectively. The literature shows HPV has more sensitivity(12) and negative predictive value to prevent misdiagnosis. Our study was based on a limited amount of cases. However, this data analysis shows that the combined cervical cytology and HPV DNA testing increases the sensitivity, significantly reducing the possibility of missed diagnosis(14).

HPV DNA testing has high sensitivity, but the results often include infections, which may increase the risk of overtreatment(15). At the same time, liquid-based cytology has high specificity. It is necessary to formulate a screening, diagnosis, and treatment plan for women with a high risk of HPV DNA according to the age of the patient, and cytology is the first choice for HPV-positive patients. The combined method has good sensitivity; it can effectively avoid missed diagnoses and misdiagnoses(16). Co-testing helps determine preinvasive or invasive lesions in the cervix. So, it is used as a sensitive diagnostic tool for screening purposes.

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