Research article Pham Ngoc Thach Medical and Pharmaceutical Journal

DOI: 10.59715/pntjmp.5.2.6

Discordance rate between cervical cytology results and HPV DNA testing and related factors among women undergoing cervical biopsy at Hung Vuong Hospital

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Abstract

Background: Cervical cancer screening strategies recommend HPV DNA testing as the primary screening method instead of PAP cytology test. However, PAP testing remains widely used, and public awareness of HPV infection remains low. According to global studies, CIN 2+ detection in PAP-negative, HPV 16/18-positive women ranges from 15% - 30%. This study aims to improve healthcare professionals' screening practices in line with national guidelines.

Objectives: To assess precancerous and cancerous lesions in discordant PAP-HPV cases and identify factors associated with discrepancies between PAP and HPV test results.

Methods: A retrospective cross-sectional study was conducted on 905 women who underwent cervical cancer screening with PAP testing and HPV DNA testing and had abnormal cervical biopsy results at Hung Vuong Hospital.

Results: The discordance rate between PAP and HPV was 52.4%, including PAP (-) HPV (+) 46.7% and PAP (+) HPV (-) 5.6%. Three factors were identified as significantly associated with PAP-HPV discordance in women with precancerous cervical lesions: Cervical evaluation, Vaginal inflammation and Histopathological severity of cervical lesions.

Conclusion: HPV DNA testing should be adopted as the primary screening method per national guidelines.

Keywords: PAP and HPV test discordance, primary HPV testing, cervical cancer screening.

Received: 21/02/2025
Revised: 21/03/2025
Accepted: 20/04/2026

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1. INTRODUCTION

Cervical cancer (CC) is one of the most common cancers among women, particularly in developing countries, with a high mortality rate. According to GLOBOCAN 2022, Vietnam recorded approximately 4,612 new cases and 2,517 deaths due to cervical cancer [1]. High-risk human papillomavirus (HPV) infection, particularly types 16 and 18, has been identified as the primary cause of cervical cancer [2]. HPV DNA testing is highly reliable and recommended as the primary screening method for early detection of cervical cancer risk by global guidelines and the Vietnamese Ministry of Health [3–6]. However, Papanicolaou (PAP) testing remains widely used due to its lower cost and the established habits of healthcare professionals and patients. Studies indicate that the detection rate of CIN 2+ lesions in women with negative PAP results but positive high-risk HPV ranges from 15% to 30% [7–10]. This study, conducted at

Hung Vuong Hospital, aims to evaluate the discordance rate between PAP and high-risk HPV testing, providing insights to enhance cervical cancer screening strategies in alignment with national and international recommendations.

2. MATERIALS AND METHODS

2.1. MATERIALS

Women undergoing gynecological examinations had cervical cancer screening tests (PAP and HPV DNA) and abnormal cervical biopsy results at Hung Vuong Hospital.

Inclusion criteria: ≥ 25 years old, underwent first-time co-testing (liquid-based cytology and Cobas HPV DNA) within the hospital, abnormal cervical biopsy results (precancerous cervical lesions or cervical cancer).

Exclusion criteria: Only underwent PAP testing or HPV testing individually; underwent conventional PAP testing or non-Cobas HPV DNA tests.

2.2. Research Methods

Study design: A retrospective cross-sectional descriptive study

Sample size: Calculated using the formula for estimating proportions in descriptive studies.

$$n \geqslant \frac{Z^2_{1-\alpha/2}(1-p)p}{d^2}$$

Based on Schiffman's 2017 study in the United States, the discordance rate between PAP and HPV testing was p = 28.9%, with α = 0.05, Zα = 1.96, and d = 0.03 [7]. The minimum required sample size was calculated to be 878 cases. In reality, 905 cases were collected.

Sampling Method: A convenience sampling method was applied, collecting medical records from January 2020 to December 2023 until the sample size requirement was met.

Study content:

Epidemiological, obstetric and gynecological characteristics include age, parity, menstrual status, clinical characteristics during gynecological examination.

Cervical Cancer Screening Methods:

1. Cytology Test (PAP Test): ThinPrep Kit using the ThinPrep Processor T5000 system (Hologic), sample was analyzed at the Pathology Department of Hung Vuong Hospital and the results were interpreted using Bethesda 2001 system, classified into two groups:

- Negative (-): < ASCUS (normal, inflammatory changes)
- Positive (+): ≥ ASCUS (ASCUS, LSIL, HSIL, ASCH, AGUS, squamous cell carcinoma, adenocarcinoma)

2. HPV Testing: Real-time PCR HPV testing using Cobas 4800 system (Roche) conducted at the Laboratory Department of Hung Vuong Hospital. The results were classified into two groups:

- Negative (-): No HPV detected or only low-risk HPV types
- Positive (+): Detection of 12 high-risk HPV types: HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59

Cervical Histopathology (Gold Standard): Patients with abnormal colposcopy findings were biopsied, and histopathological results were reviewed by pathologists at Hung Vuong Hospital.

Discordance between cytology and HPV DNA testing in detecting cervical cancer lesions was defined as: PAP (-) HPV (+) and PAP (+) HPV (-)

2.3. Data Processing

Data was entered by Microsoft Excel and analyzed by Stata 14.0.

2.4. Ethics

The study was reviewed and approved by the Ethics Committee of Pham Ngoc Thach University of Medicine and Hung Vuong Hospital before implementation.

3. RESULTS

3.1. Epidemiological and Obstetric-Gynecological characteristics of the study population

Table 1. Epidemiological and Obstetric-Gynecological characteristics of the study population

Characteristics	n = 905	%
Age	39 ± 9.2	
Age group		
25-29	147	16.2
30-39	333	36.8
40-49	295	32.6
≥ 50	130	14.4
Parity		
0	138	15.3
1	238	26.3
2 and more	529	58.4
Menstrual status		
Menstruating	819	90.5
Menopausal	86	9.5
Cervical evaluation		
Smooth cervix	698	77.1
Suspected lesion	90	9.9
Ectropion	104	11.5
Cervical polyp	13	1.5
Vaginitis		
Present	121	13.4
Absent	784	86.6

The average age was 39 ± 9.2 years. The majority of participants were aged 30-39 years (36.8%) and 40-49 years (32.6%). Most women had experienced two or more pregnancies (58.4%), and the majority were still menstruating (90.5%). A smooth cervix was the most common finding (77.1%), and there was a significant difference in the prevalence of vaginitis (13.4%) compared to those without (86.6%).

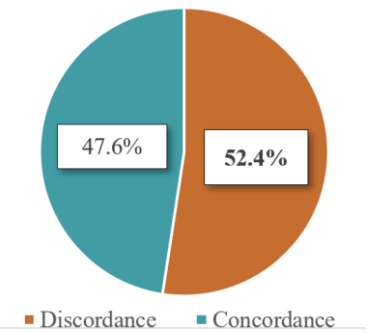

Chart 1. Discordance rate between PAP and HPV tests

Table 2. Co-Testing Results

Test Characteristics		n = 905	%	
Concordant	PAP (+) HPV (+)	382	42.2	47.6
	PAP (-) HPV (-)	49	5.4	
Discordant	PAP (-) HPV (+)	423	46.7	52.4
	PAP (+) HPV (-)	51	5.6	

The concordance rate between PAP and HPV tests was 47.6%, including PAP (+) HPV (+) (42.2%) and PAP (-) HPV (-) (5.4%). The discordance rate was 52.4%, consisting of PAP (-) HPV (+) (46.7%) and PAP (+) HPV (-) (5.6%).

Table 3. Correlation Between PAP and HPV Results

PAP \ HPV	HPV (-)	HPV (+)	Total
PAP (-)	49	423	472
PAP (+)	51	382	433
• ASCUS	27	196	223
• ASCH	4	23	27
• AGUS	1	7	8
• LSIL	18	103	121
• HSIL	1	51	56
• Squamous Cell Carcinoma	0	2	2
Total	100	805	905

If **HPV testing alone** were used as the **primary screening method**, **11% (100/905) of cases** would have been missed. Conversely, if **PAP testing alone** were used, **52.2% (472/905) of cases** would have been missed. **ASCUS** (51.5%) was the most common lesion, followed by **LSIL** (**27.9%**).

Table 4. PAP Test Results Compared to Histopathology

PAP \ GPB	CIN 1	CIN 2	CIN 3	SCC	ADC	Total	p
Benign	339	73	53	6	1	472	<0.001
Abnormal						433	
• ASCUS	148	32	40	3	0	223	
• ASCH	6	6	14	1	0	27	
• AGUS	3	0	3	0	2	8	
• LSIL	83	19	18	0	1	121	
• HSIL	8	12	32	0	0	52	
• Squamous Cell Carcinoma	0	0	2	0	0	2	
Total	587	142	162	10	4	905	

Among **472 cases with negative PAP results**, **126 cases (26.7%)** were diagnosed as **CIN 2/3**, including **6 cases of squamous cell carcinoma (SCC) (1.3%)** and **1 case of adenocarcinoma (ADC) (0.2%)**. There was **statistically significant difference** between **PAP test results and histopathological findings** (**p < 0.001**).

Table 5. HPV Test Results Compared to Histopathology

HPV / GPB	CIN1	CIN2	CIN3	SCC	ADC	Total	%	p
Negative	72	10	17	1	0	100	11	0.099
Positive						805	89	
16	119	32	36	4	0	191		
18	54	9	11	1	1	76		
12 High-risk types	264	64	75	3	2	408		
≥ 2 types	78	27	23	1	1	130		
Total	587	142	162	10	4	905		

The rate of HPV-negative cases was 11% (100/905), while HPV-positive cases accounted for 89% (805/905). Among the 100 HPV-negative cases, CIN 1 accounted for 72% (72/100), while 28% (28/100) had CIN 2 or higher lesions. There was no statistically significant association between HPV test results and histopathological findings in this study (p > 0.05).

Table 6. Co-Testing Results Compared to Histopathology

Co-testing / GPB	CIN 1	CIN 2	CIN 3	SCC	ADC	Total	%
PAP (-) HPV (+)	298	68	50	6	1	423	46.74
PAP (+) HPV (-)	31	5	14	1	0	51	5.64
PAP (+) HPV (+)	217	64	95	3	3	382	42.21
PAP (-) HPV (-)	41	5	3	0	0	49	5.41
Total	587	142	162	10	4	905	100

Table 7. PAP Test and HPV Test Compared to Histopathology

TEST / GPB	CIN 1	CIN 2	CIN 3	SCC	ADC	Total
PAP (-)	339	73	53	6	1	472 (52.2%)
		73	53	6	1	133 (14.7%)
HPV (-)	72	10	17	1	0	100 (11%)
		10	17	1	0	28 (3.1%)

The negative rate was higher for PAP tests compared to HPV tests. Using a threshold of CIN 1 or higher, PAP-negative cases accounted for 52.2%. The PAP test missed cervical precancerous lesions 4.7 times more than HPV test. When considering CIN 2+ lesions, the PAP-negative rate was 14.7%, confirming that PAP testing failed to detect CIN 2+ cases 4.7 times more than HPV testing.

Table 8. Univariate Analysis of Factors Associated with Discordance Between PAP and HPV Tests

Characteristics	Discordance				OR	95% CI	p
	Yes n	%	No n	%			
Age					0.99	0.98 – 1.01	0.694
Age group							
25-29	76	51.7	71	48.3	1		
30-39	168	50.5	165	49.5	0.95	0.65 – 1.40	0.801
40-49	162	54.9	133	45.1	1.14	0.77 – 1.69	0.523
≥50	68	52.3	62	47.7	1.02	0.64 – 1.64	0.920
Parity							
0	62	44.9	76	55.1	1		
1	115	48.3	123	51.7	0.87	0.57 – 1.33	0.525
≥ 2	254	48	275	51	0.88	0.6 – 1.29	0.518
Menstrual status							
Menstruating	420	52.3	391	47.7	1		
Menopausal	46	53.5	40	46.5	1.05	0.67 – 1.64	0.828
Cervical evaluation							
Smooth cervix	430	61.6	268	38.4	1		
Suspected lesion	12	13.3	78	86.7	0.1	0.05 – 0.18	< 0.001
Ectropion	27	26	77	74	0.22	0.14 – 0.35	< 0.001
Cervical polyp	5	38.5	8	61.5	0.39	0.13 – 1.2	0.101
Vaginitis							
Absent	396	50.5	388	49.5	1		
Present	78	64.5	43	35.5	1.78	1.19 – 2.65	0.005
Histopathology							
CIN 1	329	56.1	258	43.9	1		
CIN 2	73	51.4	69	48.6	0.83	0.57 – 1.2	0.319
CIN 3	64	39.5	98	60.5	0.51	0.36 – 0.73	<0.001
SCC	7	70	3	30	1.83	0.47 – 7.15	0.385
ADC	1	25	3	75	0.26	0.03 – 2.53	0.246

Table 9. Multivariate Analysis of Factors Associated with Discordance Between PAP and HPV Tests

Characteristics	OR	95% CI	p
Cervical evaluation			
Smooth cervix	1		
Suspected lesion	0.1	0.05 – 1.20	< 0.001
Ectropion	0.19	0.12 – 0.31	< 0.001
Cervical polyp	0.36	0.11 – 1.1	0.075
Vaginitis			
Absent	1		
Present	1.58	1.02 – 2.44	0.047
Histopathology			
CIN 1	1		
CIN 2	0.86	0.58 – 1.29	0.469
CIN 3	0.53	0.36 – 0.78	0.001
SCC	2.19	0.46 – 10.41	0.325
ADC	0.18	0.02 – 1.70	0.133

Smooth cervix had a significantly higher risk of discordance between PAP and HPV compared to suspicious cervical lesions and ectropion. Vaginitis increased the likelihood of discordance compared to women without vaginitis (OR = 1.58; 95% CI: 1.02 – 2.44; $p < 0.05$). Women with CIN 1 had a higher risk of discordance compared to those with CIN 3 (OR = 0.53; 95% CI: 0.36 – 0.78; $p < 0.05$).

4. DISCUSSION

The average age of the study population was 39 ± 9.2 years, with the 30–39 age group having the highest proportion (36.8%). This age group is at high risk for HPV infection and progress to precancerous cervical lesions. However, it should concern the prevalence of precancerous cervical lesions in the under-30 age group (16.2%), as early sexual activity and long-term HPV infection may contribute to an increased risk of disease [11]. Obstetric history shows that nearly 60% of the study population had given births at least twice. Regarding menstrual status, 9.5% of participants were in the menopausal stage. The decline of estrogen levels can cause cellular changes, leading to false-positive or false-negative results in PAP testing. According to Gilani's study (2011), the false-positive rate of PAP tests was significantly higher in postmenopausal women compared to the premenopausal group [12]. Cervical assessment showed that 77.1% of patients had a clinically smooth cervix. However, the study also recorded numerous cases of cervical suspicious lesions or severe ectropion, despite negative screening results. This highlights the importance of combining clinical examination with laboratory testing to minimize missed diagnoses. Lastly, 13.4% of the study population had lower genital tract infections, a factor that may increase the risk of false-negative PAP test results. Severe infections can obscure abnormal cells, affecting test accuracy, as demonstrated in studies by Goodman (2018) and Macios (2021). [13,14].

Discordance Between PAP and HPV Testing

[Table 2] shows that the concordance rate between PAP and HPV testing is 47.6%, including PAP (+) HPV (+) (42.2%) and PAP (-) HPV (-) (5.4%). However, the discordance rate is as high as 52.4%, primarily due to PAP (-) HPV (+) (46.7%), indicating that PAP testing has a high risk of missing cases of high-risk HPV infection. Compared to international studies, Schiffman (2017) in the U.S. reported a discordance rate of 28.9%, while Kaufman (2020) recorded 20.6%, significantly lower than the findings in this study [7,15]. The difference may be attributed to sample collection methods, population characteristics, and the criteria for defining CIN 2+ lesions instead of CIN 1+.

Analysis of the correlation between PAP and HPV results [**Table 3**] indicates that if HPV testing were used as the only screening method, 100 cases (11%) would have been missed. Among PAP (+) cases, ASCUS accounted for the majority (53%, 27/51). If only PAP testing was performed, 52.2% (472/905) of cases would have been missed, as negative PAP results would not lead to colposcopy for further disease detection.

The correlation between PAP results and histopathology [**Table 4**] reveals that using PAP as the primary screening test would have missed 52.2% (472/905) of disease cases. Among patients with negative PAP results, CIN 2+ lesions accounted for 28.2% (133/472), compared to 15.1% in Park's (2015) study and 28.6% in Ding's (2023) study. ASCUS and LSIL were primarily associated with CIN 1, while ASCH, HSIL, and squamous cell carcinoma on PAP were linked to a high rate of CIN 3.

Regarding the correlation between HPV and histopathology [**Table 5**], HPV failed to detect disease in 11% of cases. Although HPV testing does not diagnose cervical pathology as PAP, a positive HPV result leads to further evaluation via PAP or colposcopy, reducing the risk of missed diagnoses compared to PAP alone. HPV 16 had a CIN 2+ rate of 8.9%, HPV 18 had 2.7%, and other high-risk HPV types accounted for 17.9%. This suggests that high-risk HPV types beyond 16 and 18 are also significantly associated with cervical cancer, aligning with findings from Emel Yilmaz (2023), Anying Bai (2023), and Kabaca (2021), which highlighted the high CIN 3+ risk of HPV types 31, 33, 52, and 58, even in cases with negative PAP results [16–18].

When comparing co-testing results with histopathology findings [**Table 6**], the PAP (-) HPV (+) group accounted for 46.7%, whereas the PAP (+) HPV (-) group made up 5.6%. Among those who were PAP (-) HPV (+), 13.8% had CIN 2+ lesions, compared to only 1.7% in the PAP (+) HPV (-) group. This confirms that PAP testing has a higher risk of missing precancerous cervical lesions compared to HPV testing, with a miss rate 4.75 times higher than that of HPV testing [**Table 7**].

In conclusion, no single screening test has absolute accuracy. Effective cervical cancer screening requires a combination of clinical examination, PAP testing, HPV DNA testing, and colposcopy when necessary. This integrated approach optimizes early lesion detection and minimizes the risk of missed cervical cancer cases.

Factors Associated with the Discordance Rate Between PAP and HPV Testing

The study identified that the discordance between PAP and HPV testing especially stems from the high sensitivity of HPV testing and the high false-negative rate of PAP testing. HPV testing detects the

presence of HPV DNA even before abnormal cellular changes occur in the cervix. This allows HPV testing to have higher sensitivity than PAP testing in identifying cervical cancer risk. However, HPV testing has lower specificity. According to Binnicker, the sensitivity of HPV testing in detecting CIN 2+ lesions is 91.4%, but its specificity is only 31.2% [19]. This means that HPV testing helps detect early latent lesions but may cause false-positive results, leading to a high discordance rate. On the other hand, PAP testing has a risk of missing lesions, with a false-negative rate ranging from 30% to 87%, according to a Cochrane study [20]. In this study, the false-negative rate of PAP testing was 52.2% (for CIN 1+) and 14.7% (for CIN 2+). Possible causes include: (1) Inadequate sample collection, particularly for lesions located deeply within the cervical canal, (2) A low number of abnormal cells, making detection difficult, and (3) Confounding factors such as infections, blood contamination, or errors in sample preservation.

Multivariate analysis **[Table 9]** identified three key related factors influencing discordance between the two tests:

- Smooth cervix had a 10 times higher risk of discordant results compared to cervices with suspected severe lesions (OR = 0.1; 95% CI: 0.05–1.20; $p < 0.001$) and 5.26 times higher risk compared to ectropion cervices (OR = 0.19; 95% CI: 0.12–1.31; $p < 0.001$).
- Vaginal infection: The risk of discordance was 1.58 times higher in women with vaginal infections compared to those without (OR = 1.58; 95% CI: 1.02–2.44; $p < 0.05$). Infections can obscure abnormal cells, leading to false-negative PAP results.
- Histopathological findings: CIN 1 lesions had 1.9 times higher discordance risk compared to CIN 3 (OR = 0.53; 95% CI: 0.36–0.78; $p < 0.05$). Milder lesions may not be pronounced enough for PAP testing to detect.

These associated factors align with findings from Goodman (2018) and Macios (2021) [13,14].

Strengths and Limitations: This study has a large sample size (905 cases), ensuring reliability and representativeness. Data management was well-executed, minimizing errors. However, the cross-sectional design does not establish causality, the retrospective nature may lead to missing key information, and some confounding factors were not fully controlled.

5. CONCLUSION

The high discordance rate between PAP and HPV testing highlights the necessity of implementing HPV DNA testing as the primary screening method instead of PAP in healthcare facilities. While HPV testing offers significant sensitivity, cytology remains a crucial role in the triage of HPV-positive cases. The transition to HPV-based screening is aligned with international trends; however, combining both tests enhances early detection and improves specificity.

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