MEDS Clinical Medicine (2024)
Clausius Scientific Press, Canada

DOI: 10.23977/medsc.2024.050408
ISSN 2616-1907 Vol. 5 Num. 4

A Clinical Evaluation of the HPV Antigen Rapid Test for Early Detection of Cervical Cancer

Zhang Lei[1,*], Yang Feng[2], Zhu Junzhe[3]

[1]Zhejiang Gongshang University, Hangzhou, Zhejiang, 310018, China
[2]Community Health Service Center, Yipeng Street, Qiantang District, Hangzhou, Zhejiang, 310000, China
[3]Wenzhou Medical University, Wenzhou, Zhejiang, 310000, China
[*]Corresponding author: zhanglei@zjgsu.edu.cn

Keywords: HPV, Cervical Cancer, Rapid Test, Diagnostics

Abstract: Cervical cancer remains a pressing global health concern. The primary cause of this malignancy is infection with high-risk human papillomavirus (HPV) strains, primarily HPV-16 and HPV-18, which account for approximately 70% of all cases. The development of effective prevention and treatment strategies depends on the availability of reliable HPV detection methods. In this study, we evaluated the diagnostic performance of the HPV antigen rapid test using a cohort of 140 clinical specimens, comparing it against PCR assays. The rapid test demonstrated high accuracy, with a sensitivity of 95%, specificity of 99.2% and overall accuracy of 98.6%. These findings highlight the potential of the HPV antigen rapid test to enhance HPV screening and facilitate early diagnosis, thereby contributing to more effective global management and prevention of cervical cancer. In summary, the HPV Antigen Rapid Test Cassette emerges as a valuable tool for HPV screening and cervical cancer prevention. Its high sensitivity and specificity, combined with the ability to provide rapid results, make it particularly beneficial for point-of-care testing in resource-limited environments.

1. Introduction

Cervical cancer is a significant health concern for women worldwide, ranking as the fourth most prevalent cancer globally. However, the burden of this disease varies considerably across different regions, with notable geographic disparities in both incidence and mortality rates.[1] The highest burden of cervical cancer is often borne by developing nations, particularly those in regions such as Sub-Saharan Africa, Latin America and parts of Asia. These areas frequently lack comprehensive screening and vaccination programs, contributing to their elevated rates of this disease. Conversely, developed countries with established robust screening protocols have experienced substantial declines in both the incidence and mortality of cervical cancer.[2]

The primary etiological factor underlying the development of cervical cancer is recognized as human papillomavirus (HPV) infection. This non-enveloped DNA virus, belonging to the Papillomaviridae family, encompasses over 200 identified types.[3] Of the over 200 identified HPV types, at least 14 high-risk varieties are considered oncogenic. Among these, HPV-16 and HPV-18

are the most significant, jointly accounting for approximately 70% of all cervical cancer cases.[4] Effective prevention and treatment strategies for cervical cancer hinge on a comprehensive understanding of the pivotal role played by human papillomavirus (HPV). This virus is primarily transmitted through direct skin-to-skin or skin-to-mucosa contact, with sexual activity, including vaginal, anal or oral intercourse, being the most common mode of transmission for individuals with an active infection.[5] Paramount clinical significance lies in the development of a streamlined, reliable and user-friendly screening method for the routine identification of human papillomavirus (HPV) genotypes 16 and 18.

The gold-standard approach for detecting human papillomavirus (HPV) relies on nucleic acid testing techniques, primarily encompassing Polymerase Chain Reaction (PCR) detection, DNA hybridization assays and RNA-based detection methods.[6] Acclaimed for its exceptional sensitivity and specificity, Polymerase Chain Reaction (PCR) technology accurately identifies a diverse range of human papillomavirus (HPV) genotypes, solidifying its standing as the gold standard for HPV diagnosis. However, despite its outstanding diagnostic performance, this method necessitates specialized laboratory equipment and technical expertise, entails a lengthier testing timeline and incurs relatively higher costs.[7] DNA hybridization assays simultaneously detect multiple human papillomavirus (HPV) types and provide objective, accurate results. However, these assays require higher specimen quality and exhibit slightly lower sensitivity compared to Polymerase Chain Reaction (PCR) methodology. Conversely, RNA-based detection directly assesses the transcriptional activity of HPV, reflecting the virus's oncogenic potential. Nonetheless, this approach has notable limitations, including the complexity of the testing procedure, stringent specimen integrity requirements and a prolonged turnaround time.[8]

In addition to the gold-standard detection methods, clinical practice has widely embraced several other prevalent human papillomavirus (HPV) testing modalities. These include cytological examination (e.g., liquid-based cytology) and colloidal gold rapid testing. Cytological screening, while straightforward and cost-effective, demonstrates relatively lower sensitivity and relies on subjective human interpretation, which introduces a degree of variability. Conversely, colloidal gold rapid testing offers a user-friendly and expedited screening option, providing results within 15-20 minutes without the need for specialized laboratory facilities.[9]

This study aims to evaluate the performance of the HPV Antigen Rapid Test Cassette developed by Hangzhou AllTest Biotech Co., Ltd, focusing on its sensitivity, specificity, accuracy and applicability in clinical and laboratory settings. By comparing its performance to PCR assays, we seek to determine its potential role in enhancing HPV screening and early diagnosis, ultimately contributing to better management and prevention of HPV-related diseases, particularly cervical cancer. This evaluation is critical, as early detection and timely intervention are pivotal in reducing the morbidity and mortality associated with cervical cancer worldwide.

2. Materials and Method

The research study utilized a total of 140 human cervical swab specimens collected from a clinically relevant population. For optimal specimen collection, it is recommended to use the swab provided by the kit manufacturer. The sampling technique involved inserting the swab into the endocervical canal, gently rotating it for approximately 10 seconds at the cervicosquamous junction or transition zone and then carefully removing the swab. The collected specimens were stored at 4 ℃ and subsequently divided into two cohorts for analysis in a biosafety laboratory setting.

(1) Rapid Test

The HPV Antigen Rapid Test Cassette (Cervical Swab) is a qualitative, lateral flow immunoassay developed for the detection of human papillomavirus (HPV) antigen in human cervical swab

specimens. This diagnostic test operates based on the specific interaction between anti-HPV antibodies and the target HPV antigen present within the specimen. Specifically, the test strip is coated with anti-HPV antibodies in the test line region and during the testing process, the HPV antigen in the specimen reacts with the anti-HPV antibody-coated recombinant protein particles on the test strip. As the specimen migrates through the membrane, the antibody-antigen complex is then captured by the immobilized anti-HPV antibodies. The appearance of a colored line in the test line region indicates a positive result for HPV infection, while the absence of this line signifies a negative result.

According to the manufacturer's instructions, the testing procedure involved adding 8 drops (approximately 450 μL) of extraction buffer into the tube. The swab was then immediately added to the extraction tube and agitated vigorously 15 times. The swab was left to soak in the extraction tube for 1 minute. It was pressed against the side of the tube and the bottom of the tube was squeezed while removing the swab to ensure that most of the liquid stayed in the tube, after which the swab was discarded. Next, the dropper tip was fitted on top of the extraction tube, the test cassette was placed on a clean, level surface and three drops of the solution (approximately 100 μL) were added to the specimen well(s). The timer was started and the colored line(s) were waited for. The result was read at 15 minutes.

(2) PCR

The detection of human papillomavirus (HPV) types 16 and 18 is commonly performed using a polymerase chain reaction (PCR)-based molecular assay. The underlying principle of this method is the selective amplification of unique genomic sequences that are specific to these high-risk HPV genotypes. Typically, the workflow involves extracting total genomic DNA from a clinical specimen, such as a cervical cytological specimen or biopsy and then utilizing oligonucleotide primer pairs targeting the unique DNA sequences of HPV 16 and 18 in the PCR reaction. The PCR thermal cycling process, involving repeated cycles of DNA denaturation, primer annealing and DNA synthesis, leads to the exponential amplification of the target HPV 16 and 18 sequences. The amplified PCR products are then analyzed using various detection methods, such as gel electrophoresis, real-time PCR or DNA sequencing, to provide definitive identification of the HPV types.

3. Results

In this study, the performance of an HPV antigen rapid test (using cervical swab specimens) was compared with PCR detection. Among the 140 clinical specimens tested, 20 were positive by PCR, of which 19 were also detected as positive by the HPV antigen rapid test, with only 1 false negative. In the 120 PCR-negative specimens, the HPV antigen rapid test identified 119 as negative, with only 1 false positive. Based on these findings (Shown in Table 1), the relative sensitivity of the HPV antigen rapid test was calculated to be 95% (95% CI: 75.1% - 99.9%), the relative specificity was 99.2% (95% CI: 95.4% - 99.9%) and the overall accuracy was 98.6% (95% CI: 94.9% - 99.8%).

Table 1: Performance Characteristics of HPV Antigen Rapid Test.

Method		PCR		Total Results
HPV Antigen Rapid Test Cassette	Results	Positive	Negative	
	Positive	19	1	20
	Negative	1	119	120
Total Results		20	120	140

Relative Sensitivity: 95% (95%CI*: 75.1%~99.9%)
Relative Specificity: 99.2% (95%CI*: 95.4%~>99.9%)
Accuracy: 98.6% (95%CI*: 94.9%~99.8%)
*: Confidence Intervals

Assessment of intra-assay consistency involved 10 repetitions of three specimen types: negative, weakly positive and moderately positive. The assay accurately detected both negative and positive outcomes in over 99% of cases, signifying high repeatability and dependability within individual assay sessions.

For inter-assay consistency, 10 separate experiments were conducted using the same specimen types. Over a three-day span, three batches of the HPV Antigen Rapid Test Cassette (Cervical Swab) were evaluated. The specimens were accurately distinguished in over 99% of instances, demonstrating robust precision across multiple assay sessions.

The investigation of cross-reactivity involved testing with 17 different microorganisms at a concentration of 10^7 CFU/test using the HPV Antigen Rapid Test Cassette (Cervical Swab). All 17 microorganisms, including *Salmonella typhi*, *Trichomonas vaginalis*, *Staphylococcus aureus*, *Acinetobacter spp.*, *Neisseria catarrhalis*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Escherichia coli*, *Gardnerella vaginalis*, *Chlamydia trachomatis* and *Ureaplasma urealyticum*, showed no reaction, which indicates the test's high specificity for the target pathogen.

4. Discussion

When diagnosed, cervical cancer is one of the most successfully treatable forms of cancer, provided that it is detected early and managed effectively.[10] Even cancers diagnosed at later stages can be controlled with appropriate treatment and palliative care.[11] Due to the long latency period from infection to invasive cancer, the WHO recommends initiating cervical cancer screening at age 30 for the general population and at age 25 for women living with HIV. [12]

However, there are some limitations to consider. The specimen size in this study was relatively small, which should be considered when interpreting the results. Future studies should aim to collect larger and more representative specimen data to enhance the statistical power and reliability of the findings. Additionally, the study did not compare different sampling methods. Employing various sampling techniques, such as random sampling, stratified sampling or cluster sampling, could provide valuable insights into how the choice of sampling technique affects the results. Comparing these different strategies would strengthen the study design and help researchers better understand the potential biases or limitations associated with the sampling methods used.

The HPV Antigen Rapid Test Cassette offers several advantages in clinical applications compared to the gold standard PCR-based detection method. Its lateral flow immunoassay format provides a more convenient and accessible screening tool, particularly in resource-limited settings or primary care facilities where access to specialized PCR equipment may be restricted. The simplified workflow and rapid turnaround time (typically 15-30 minutes) of the rapid test enable prompt initial screening, allowing for timely medical management and referral of high-risk patients for further management. Additionally, the relatively low cost per specimen of the rapid test compared to PCR-based assays enhances its feasibility for large-scale population-based screening programs, facilitating broader coverage and improved healthcare accessibility.

While the rapid test offers advantages in convenience and cost-effectiveness for initial screening, the PCR-based method remains the gold standard for comprehensive HPV diagnosis, genotyping and risk stratification. PCR techniques provide superior analytical sensitivity and the ability to detect a broader range of high-risk HPV genotypes, which is crucial for accurate diagnosis and risk assessment. The complementary use of the rapid test for primary screening, followed by confirmatory PCR testing for positive cases, can establish a more robust and holistic clinical management approach. This integrated strategy leverages the strengths of both methods, optimizing the trade-off between accessibility, cost-effectiveness and diagnostic accuracy, ultimately leading to more comprehensive and personalized patient care.

Looking ahead, future development of HPV detection technologies is likely to focus on expanding the range of genotypes covered, improving the sensitivity and specificity of both rapid and PCR-based assays and exploring emerging approaches such as microfluidic-based and immunochromatographic techniques. The integration of novel technologies, such as lab-on-a-chip platforms and advanced signal detection methods, has the potential to further enhance the performance, portability and scalability of HPV detection systems. These innovative advancements lead to more accurate, efficient and widely accessible HPV screening and diagnosis, ultimately contributing to more effective prevention and management of cervical cancer and other HPV-associated diseases.

Regarding the future development of HPV detection technologies, improving the patient experience and expanding the accessibility of these critical diagnostic tools is essential. A key focus should be on developing more patient-centered sampling methods beyond traditional cervical sampling. Innovative non-invasive sampling techniques, such as self-collected vaginal or urine specimens, could significantly improve the convenience and acceptability of HPV testing for female patients. These alternative sampling methods have the potential to increase screening uptake, especially among underserved populations who may face barriers to accessing traditional pelvic examinations. By empowering women to collect their own specimens in the privacy of their homes, these non-invasive approaches help overcome cultural, logistical and psychological obstacles that may hinder routine cervical cancer screening. Regarding specimen selection, urine is more readily accepted[13], but challenges such as low HPV viral load and pathogen contamination must be addressed. This necessitates the development of more precise sampling methods, as well as improved specimen preparation and storage techniques.

In addition to expanding sampling options, future HPV detection technologies should also prioritize the development of more user-friendly and integrated diagnostic devices. The integration of rapid testing capabilities with mobile or handheld platforms could bring HPV screening closer to the point of care, enabling immediate risk assessment and facilitating timely clinical decision-making. Such portable and automated solutions, potentially coupled with cloud-based data management and telemedicine capabilities, could significantly improve the reach and accessibility of HPV testing, particularly in resource-limited or remote settings.

Furthermore, as the landscape of high-risk HPV genotypes continues to evolve, next-generation HPV detection assays should aim to broaden their coverage and enhance the specificity of identifying clinically relevant viral strains. Expanding the panel of HPV genotypes targeted by these tests, while maintaining or improving overall sensitivity, will be crucial for more comprehensive risk assessment and personalized management strategies.

By addressing the needs of both healthcare providers and female patients, the future development of HPV detection technologies should aim to create a seamless and empowering healthcare experience. Integrating user-friendly sampling methods, portable diagnostic platforms and expanded genotypic coverage will be instrumental in increasing screening uptake, enabling earlier detection and facilitating more effective clinical interventions to combat the burden of cervical cancer and other HPV-related diseases.

5. Conclusion

In summary, the HPV Antigen Rapid Test Cassette emerges as a valuable tool for HPV screening and cervical cancer prevention. Its high sensitivity, specificity and rapid result capability make it particularly useful for point-of-care testing, especially in low-resource settings. While further research is needed to fully understand its performance across diverse populations and clinical scenarios, the current study provides strong evidence of its efficacy and potential impact on public

health. Integrating such rapid tests into existing screening programs could enhance early detection and intervention, ultimately alleviating the global burden of HPV-related diseases.

References

[1] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018 68 (6):394–424.
[2] Stelzle D, Tanaka LF, Lee KK, et al. Estimates of the global burden of cervical cancer associated with HIV. Lancet Glob Health 2021; 9(2):e161-e169.
[3] Bzhalava D, Guan P, Franceschi S, Dillner J, Clifford G (October 2013). "A systematic review of the prevalence of mucosal and cutaneous human papillomavirus types". Virology 445 (1–2): 224–231.
[4] Bouvard V, Baan R, Straif K, Grosse Y, Secretan B, Ghissassi FE, Benbrahim-Tallaa L, Guha N, Freeman C, Galichet L. A review of human carcinogens—part B: biological agents. Lancet Oncol 2009;10(4):321–322.
[5] Petca A, Borislavschi A, Zvanca ME, Petca RC, Sandru F, Dumitrascu MC. Non-sexual HPV transmission and role of vaccination for a better future (Review). Exp. Ther. Med. 2020; 20: 186.
[6] Chen L, Watanabe K, Haruyama T, Kobayashi N. Simple and rapid human papillomavirus genotyping method by restriction fragment length polymorphism analysis with two restriction enzymes, J. Med. Virol. 85 (2013) 1229–1234.
[7] Kundrod KA, Jeronimo J, Vetter B, Maza M, Murenzi G, Phoolcharoen N, et al. Toward 70% cervical cancer screening coverage: Technical challenges and opportunities to increase access to human papillomavirus (HPV) testing. PLOS Glob Public Health 2023;3:e0001982.
[8] Lugo-Trampe A, Trujillo-Murillo KdC, Rodriguez-Sanchez IP, Barboza-Cerda MdC, Lugo-Trampe JdJ, Hernandez-Ramirez LC, Canseco-Avila LM, Espinoza-Ruiz M, Dominguez-Arrevillaga S, Delgado-Enciso I. A PCR-RFLP method for typing human papillomavirus type 16 variants. J Virol Methods 2013; 187(2):338–344.
[9] Ji T, Xu X, Wang X, Cao N, Han X, Wang M, Chen B, Lin Z, Jia H, Deng M, Xia Y, Guo X, Lei M, Liu Z, Zhou Q, Chen G. Background-free chromatographic detection of sepsis biomarker in clinical human serum through near-infrared to near-infrared upconversion immunolabeling. ACS Nano 2020; 14(12):16864–16874.
[10] Katki HA, Kinney, WK, Fetterman B, Lorey, T., Poitras, N.E., Cheung, L, Castle, P.E. (2011). Cervical cancer risk for women undergoing concurrent testing for human papillomavirus and cervical cytology: A population-based study in routine clinical practice. Lancet Oncology 2011; 12, 663–672.
[11] Comprehensive Cervical Cancer Control: A Guide to Essential Practice. 2nd edition. Geneva: World Health Organization; 2014. Preface. Available from: https://www.ncbi.nlm.nih.gov/books/NBK269613/
[12] WHO guideline for screening and treatment of cervical pre-cancer lesions for cervical cancer prevention, second edition. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO.
[13] Vorsters A, Micalessi I, Bilcke J, Ieven M, Bogers J, Damme P. Detection of human papillomavirus DNA in urine. A review of the literature. Eur J Clin Microbiol Infect Dis. 2012; 31(5): 627-640.