REVIEW ARTICLE

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Cite as: Gonzalez M. Detection of human papillomavirus in urine. Quality assessment of meta-analyses. Rev. Per. Ginecol. Obstet. 2025;71(4). DOI: https://doi.org/10.31403/rpgo.v71i2822

Detection of human papillomavirus in urine. Quality assessment of meta-analyses

Detección del virus del papiloma humano en orina. Evaluación de calidad de los metaanálisis

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DOI: https://doi.org/10.31403/rpgo.v71i2822

ABSTRACT
A methodological quality assessment was conducted on meta-analyses investigating the detection of human papillomavirus (HPV) in urine samples. A systematic review of meta-analyses was performed using the search term "HPV in urine" in the PubMed, Embase, and Scopus databases. After removing duplicates and studies unrelated to the topic or not meta-analyses during the title and abstract screening, seven studies were included for analysis. The methodological quality of the selected meta-analyses was assessed using the AMSTAR 2 tool, and all were classified as critically low quality. Although the results of these meta-analyses suggest that HPV DNA detection in urine could be useful for identifying cervical HPV infection, the significant methodological limitations observed substantially reduce confidence in their conclusions. Overall, the assessment showed that overall confidence in the results was critically low
Keywords: Mass Screening, Follow-Up Studies, Quality Control, Human Papillomavirus Viruses, Cervix Uteri, Urine

RESUMEN
Se realizó una evaluación de la calidad metodológica de los metanálisis que han investigado la detección del virus del papiloma humano (VPH) en muestras de orina. Para ello, se llevó a cabo una revisión sistemática de metanálisis identificados mediante la búsqueda del término "HPV in urine" en las bases de datos PubMed, Embase y Scopus. Tras eliminar duplicados y excluir estudios no relacionados con el tema o que no correspondían a metanálisis durante el cribado por título y resumen, se incluyeron siete estudios para su análisis. La calidad metodológica de los metanálisis seleccionados se evaluó mediante la herramienta AMSTAR 2, y todos fueron clasificados como de calidad críticamente baja. Aunque los resultados de estos metanálisis sugieren que la detección de DNA del VPH en orina podría ser útil para identificar infección cervical por este virus, las importantes limitaciones metodológicas observadas reducen de manera sustancial la confianza en sus conclusiones. En conjunto, la evaluación realizada indica que la evidencia disponible mostró que la confianza general en los resultados fue críticamente baja.
Palabras clave: Cribado, Estudios de seguimiento, Control de Calidad, Papillomavirus Humano, Cuello del Útero, Orina.

Introduction

In 2022, the estimated incidence of cervical cancer worldwide was 662,301 cases, with 348,874 deaths that same year[1]. Screening plays a very important role among public health strategies aimed at reducing mortality from this disease. Of the tests available for this purpose, human papillomavirus (HPV) DNA tests have proven to be the most accurate, reproducible, and have the highest sensitivity (95%) for cervical intraepithelial neoplasia 3[2], making them the recommended tests for the early detection of this neoplasia. However, cervical cancer screening has low coverage in some regions, primarily in remote areas with limited access to health services[3]. In this context, the possibility arises for women to collect a urine sample for human papillomavirus testing—a more accessible and acceptable method[4]—and to be referred to a referral center equipped with technology for evaluating the virus's DNA.

Systematic reviews and meta-analyses are part of secondary research, which involves examining the available evidence on a specific health intervention in order to answer research questions[5].

The objective of this study is to assess the quality of meta-analysis studies that examine human papillomavirus testing in urine, using the AMSTAR 2 tool (A Measurement Tool to Assess Systematic Reviews), which enables the critical evaluation of systematic reviews in health that have included randomized, non-randomized, or mixed-design studies[6].

Information search methodology

This is a quality assessment study. In January 2026, a systematic review of meta-analysis studies was conducted using the search terms "HPV in urine" in the PubMed, Embase, and Scopus databases, applying the filters "Meta-Analysis," "Systematic Review," and "Review," respectively. The retrieved articles were screened by title and abstract independently by another reviewer. The selection criteria required that the studies be meta-analyses and that they evaluate the detection of human papillomavirus in urine using molecular techniques. It was agreed that in case of disagreement, the full text of the article would be read to reach a decision. For the articles mutually selected in this screening, the author proceeded individually, defining—based on the selected full-text articles—the data extraction for the meta-analysis for qualitative analysis and its quality assessment using the AMSTAR 2 assessment tool.

Ethical considerations: This study is considered a non-risk research study. The data were obtained from published articles; no individuals were evaluated.

Theme development

The database search identified 95 articles (Figure 1). Duplicate titles were initially excluded. The remaining abstracts were selected based on whether they were meta-analyses and their relevance to the objective of this study. Finally, seven meta-analyses were selected for full review by the author, all of which were approved for analysis. Table 1.

In the meta-analysis by Pathak et al[7], the detection of human papillomavirus DNA in urine was compared with detection in the cervix. Most of the studies included in the analysis used the polymerase chain reaction (PCR) test. A meta-regression revealed an increase in sensitivity when urine samples were collected during the first voiding compared to random or midstream samples (p=0.004).

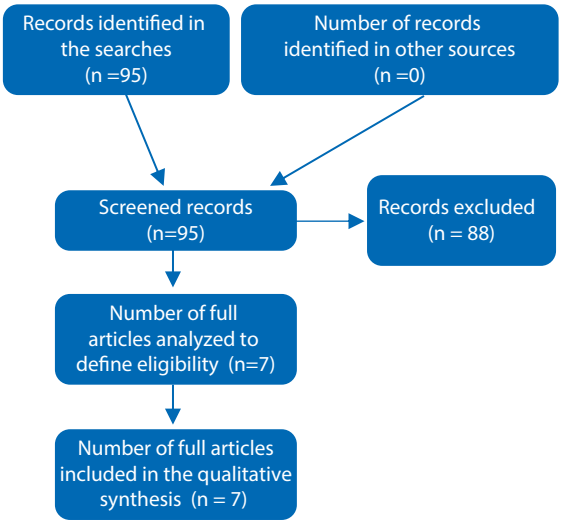

Figure 1. Information flow through the different phases of the systematic review.

Table 1. Results of diagnostic accuracy measures for high-risk HPV and high-grade CIN in meta-analyses evaluating studies on human papillomavirus DNA testing in urine.

Author	Year	Included Studies	Comparison	Sensitivity % (95%CI)	Specificity % (95%CI)	PLR (95%CI)	NLR (95%CI)
Pathak et al[7]	2014	14	Cervical samples	77 (68-84)	88 (58–97)	6.33 (1.48–27.00)	0.26 (0.16–0.41)
Bober et al[8]	2021	15	Cervical samples	78 (70–84)	89 (81–94)	6.81 (4.07–11.41)	0.25 (0.18–0.34)
Cho et al[9]	2022	21	Cervical samples	79 (72–86)	93 (89–96)	1.52 (1.33–1.75)	0.44 (0.30–0.58)
Park et al[10]	2025	15	Cervical samples	82 (78–86)	91 (87–94)	9.5 (6.3–14.3)	0.19 (0.16–0.24)
Li et al[11]	2025	15	Cervical Samples	83 (77-88)	81 (65–91)	4.37 (2.21–8.64)	0.21 (0.15–0.30)
Hsiao et al[12]	2025	21	Histopathology or colposcopy	85.2 (82.5- 87.6)	49.4 (37–61.8)	1.68 (1.32-2.16)	0.30 (0.22–0.41)
Ye et al[13]	2025	65	Cervical Samples	76 (72–80)	90 (87-92)	7.60 (6.18–9.35)	0.27 (0.23–0.32)
		35	Histopathology CIN 2+	79 (72–84]	58 (50–65)	1.88 (1.55–2.28)	0.36 (0.26–0.50)

PLR: Positive likelihood ratio. NLR: Negative likelihood ratio. CI: Confidence interval = 95%

The study by Bober et al[8] evaluated the accuracy of human papillomavirus detection in first-morning urine compared with that from the cervix. In most of the study populations, cervical cancer screening was the purpose of testing (10/15), and conventional PCR was used; however, the methods were not uniform. This study included in its meta-regression the variables of patient selection bias, purpose, timing of the sample, storage temperature, and human papillomavirus testing method to investigate possible sources of heterogeneity that were not detected.

Cho et al[9], assessed the clinical accuracy of these tests for detecting cervical intraepithelial neoplasia 2 or higher. In follow-up studies, the combined sensitivity for detecting cervical intraepithelial neoplasia grade 2 or higher was 79% (95% CI = 0.72–0.86) and 93% (95% CI = 0.89–0.96) for urine samples and physician-collected samples, respectively. There were only 3 primary screening studies in the meta-analysis. Most studies used high-risk human papillomavirus PCR assays. The meta-regression failed to show a significant effect of urine collection time, sampling device, or storage medium.

In their review of 15 studies, Park et al[10] found significant heterogeneity, particularly in terms of sensitivity and specificity (the Higgins I_2 values were 0.8218 for sensitivity and 0.8980 for specificity). Subgroup analysis indicated that urine volumes ≤20 mL showed higher sensitivity compared to those >20 mL, although this was based on a limited number of studies.

Li et al.[11], in 15 cross-sectional studies involving 3,665 participants, all with abnormal results on cervical cancer screening tests, evaluated the diagnostic performance of two self-collection methods (vaginal and urine) and physician-collected samples for the detection of high-risk human papillomavirus. The area under the curve (AUC) for urine samples was 0.88 (95% CI: 0.85–0.91). For lesions greater than CIN 2, the urine sample showed a sensitivity of 95% (95% CI: 0.91–0.97) and a specificity of 62% (95% CI: 0.31–0.86), and the AUC was 0.95 (95% CI: 0.93–0.97). Most studies used colposcopy and histological diagnosis as the gold standard.

Hsiao et al[12] conducted studies evaluating the diagnostic accuracy of HPV testing using urine samples collected by participants during screening for high-grade squamous intraepithelial lesions or more advanced lesions, using DNA- or RNA-based HPV tests. Most of the studies were prospective (17 out of 21). In 19 publications, the effectiveness of DNA-based tests for high-risk HPV in urine samples was examined, while 2 investigated the performance of RNA-based tests. A pooled sensitivity of 85.2% and a pooled specificity of 49.4% were obtained from the analysis of subgroups of manuscripts that used DNA-based tests. For studies evaluating RNA tests, sensitivity was 44.7% (31.4%–58.8%) and specificity was 68.9% (31.9%–91.3%).

Ye et al[13], in addition to other analyses, present data from 13 studies (2,426 participants) that used questionnaires or interviews, revealing a high degree of patient acceptance and comfort with urine sample collection. The combined acceptability was 95% (95% CI: 74%–99%), with a combined comfort level of 98% (95% CI: 95%–99%) and a satisfaction rate with the method of 95% (95% CI: 47%–100%). Most women expressed a preference for urine collection over other methods, with a combined estimate of 69% (95% CI: 54%–82%).

The AMSTAR 2[6] assessment tool applied to these seven meta-analyses revealed shortcomings in the appropriateness of the meta-analysis methods used for the statistical pooling of results; there was no reporting or justification of excluded studies; and the risk of bias in individual studies was not taken into account when interpreting or discussing the review's results. There were also specific flaws in each study, resulting in the overall confidence in the review results being rated as critically low.

Cervical cancer is a preventable disease; however, its incidence and mortality remain high in low- and middle-income countries[14,15]. Its primary cause is infection with a high-risk type of human papillomavirus[16]. Detection of these viruses has become the recommended screening test given its high sensitivity, which makes it superior to the Pap test for detecting cervical intraepithelial neoplasia grade 2/3 or higher[2].

Coverage rates for screening programs remain a major barrier to realizing their benefits; even in developed countries with relatively ample healthcare resources, approximately 20% to

30% of women do not participate in cervical cancer screening programs[17]. This is due to problems with planning, organization, and resources, but also because women refuse to attend these services due to personal, educational, or cultural beliefs, compounded by the fact that most cervical cancers occur when there is low adherence to screening schedules among women who do not attend regularly or who have never undergone screening[18]. An alternative developed to address these issues is self-collection of vaginal samples for human papillomavirus detection by the woman herself. Studies comparing this procedure with samples collected by a physician found that both methods yielded similar results[19–21]. The use of self-collection has the potential to reduce barriers to testing and reach women at risk. With this strategy, due to its high acceptance, participation was significantly improved among women who did not routinely undergo cervical cancer screening and women in rural areas with limited access to health centers[22]. Additionally, among the self-administered tests is the detection of human papillomavirus in a urine sample, which is a test known and accepted by the population. With this test, using the appropriate preservative and the first urine sample, highly sensitive results can be obtained that are consistent with those collected by a clinician[23], leading to greater acceptance and, consequently, better population coverage in screening programs among women who are reluctant to undergo a cervical Pap test or to collect their own vaginal sample[24,25].

The meta-analyses examining the detection of human papillomavirus in urine, which were reviewed for this study, have notable characteristics. In the review by Pathak et al.[7], the included studies did not have a uniform purpose, although most were conducted as screening tests. They varied in the timing of urine sample collection (random, first void, or midstream), the volume of urine analyzed, storage temperature, and the method used to detect the virus[8].

The meta-analyses reviewed reaffirm in their results the feasibility of detecting high-risk human papillomavirus in urine[7–13]. However, they acknowledge limitations due to heterogeneity in testing methods. Additionally, the studies were conducted in various settings, including primary and secondary care, using different platforms and testing conditions for human papillomavirus[7], and the hierarchical models (such as the bivariate model) used may be vulnerable when the number of studies is small and also when sample sizes are highly variable[7–13].

As evidenced by the AUC results in Li et al[11], the test is highly effective at correctly classifying patients based on their actual health status. However, there is considerable heterogeneity among the studies, which may limit the interpretation of the results.

In Cho et al[9], studies comparing urine-based polymerase chain reaction (PCR) tests with samples collected by a physician demonstrate similar clinical accuracy in detecting cervical intraepithelial neoplasia grade 2 or higher. This meta-analysis included primary screening studies (3 studies) and follow-up studies. It cites as a limitation the relatively small number[21] and small sample sizes (<500) of the included studies.

DNA-based HPV nucleic acid amplification testing in urine has higher sensitivity than RNA-based HPV nucleic acid amplification testing, which is not recommended for this purpose[12].

If we accept the working hypothesis that the detection of human papillomavirus in urine occurs because vaginal secretions may contain exfoliated cervical and vaginal cells infected with human papillomavirus that are washed out with the urine[26], it is expected that testing this fluid will yield lower results than those obtained through direct sample collection by the clinician or vaginal swabbing performed by the woman. This is reflected in the traditional and basic measures of the test's diagnostic value. Among these, sensitivity—defined as a test's ability to correctly classify individuals with the disease as positive[27]—is lower than that reported for human papillomavirus DNA tests performed by healthcare personnel[2], but appears to exceed that of cytology (72.9% 95% CI: 70.7–75%)[28], which is important when designing screening protocols. However, it is necessary to standardize testing conditions to more accurately evaluate the procedure in terms of validity, reliability, performance, cost, acceptance, and follow-up[27]. In this regard, Van Keer et al.[26] propose that optimized detection of HPV DNA in urine should include the following: collection of the first-morning urine in a device designed for this purpose,

prevention of DNA degradation by immediately mixing the urine with an appropriate preservation medium previously placed in the collection container, a urine sample of adequate volume, complete processing of the first-morning urine rather than centrifuged samples, and the use of human papillomavirus tests that meet the criteria for primary screening for cervical cancer[29].

Meta-analyses are important components of scientific evidence in evidence-based medicine[30]. Their number has increased, but not always their quality[31].

The AMSTAR 2 tool is an updated version of the original AMSTAR. It can be used to assess the methodological quality of systematic reviews that include both randomized and non-randomized studies of health interventions. AMSTAR 2 provides guidance for rating the overall confidence in the results of a review (high, moderate, low, or critically low) depending on the number of critical flaws and/or non-critical weaknesses[6].

The quality assessment of the meta-analyses evaluated using the AMSTAR 2 tool[6] rated the overall confidence in the meta-analysis results as critically low. This means they have more than one critical flaw and should not be relied upon to provide an accurate and complete summary of the available studies.

The limitations of this study stem from the design of the AMSTAR 2 tool in assessing the planning and conduct of reviews. As a new tool that includes non-randomized studies in systematic reviews, it is necessary to await feedback from users of the instrument to consider making modifications[6].

Conclusions

Detecting human papillomavirus in urine could improve women's participation in cervical cancer screening; however, further research is needed to optimize its effectiveness. The current assessment found that the methodological quality of the meta-analyses that evaluated the test using the AMSTAR 2 tool resulted in an overall confidence in the results of those reviews that was critically low.

Article information

Received: October 31, 2025
Accepted: January 9, 2026
Published online: March 16, 2026

Conflicts of interest: The author declares no conflicts of interest.

Funding source: Self-funded.

Statement on the use of artificial intelligence (AI): The author declares that no AI tools were used in the preparation of this article.

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