# PapilloDia – Selected References

## *Published Articles and Guidelines*

| Author(s) | Journal | Year | Title |
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| *Zhang et al.* | *Journal of International Medical Research* | *2018* | *Feasibility study of a human papillomavirus E6 and E7 oncoprotein test for the diagnosis of cervical precancer and cancer* |

**Summary:**
Objective To evaluate the clinical value of human papillomavirus (HPV) E6 and E7 oncoprotein (HPV E6/E7) detection in the early screening of cervical cancer. Methods This prospective study evaluated all patients with suspected cervical intraepithelial neoplasia (CIN) as identified by the presence of at least one positive indicator from a ThinPrep cytologic test (TCT) and/or a Hybrid Capture 2 (HC2) HPV DNA test. The levels of E6/E7 oncoproteins were determined using Western blot analysis. The diagnostic value of the HPV E6/E7 protein assay was compared with the clinical diagnosis from TCT, HC2 and the gold standard of cervical biopsy histology. Results A total of 450 patients were enrolled in the study and based on histological findings, 102 patients were diagnosed with CIN1 (22.7%), 241 with CIN2 (53.6%), 96 with CIN3 (21.3%) and 11 with squamous cell carcinoma (2.4%). For a diagnosis of CIN2+, although the sensitivity of the HPV E6/E7 assay was lower than HC2 (65.5% versus 96.6%, respectively), the specificity was higher (38.2% versus 5.9%, respectively). The sensitivity of the HPV E6/E7 assay was higher than TCT (65.5% versus 36.2%, respectively). Conclusion Measuring HPV E6/E7 oncoprotein levels is a potential new biomarker for HPV type 16.

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| *Kong et al.* | *BMC Cancer* | *2020* | *Analysis of the role of the human papillomavirus 16/18 E7 protein assay in screening for cervical intraepithelial neoplasia: a case control study* |

**Summary:**
Cervical cancer is the second-most common gynecological cancer, early screening plays a key role in the diagnosis and treatment of cervical intraepithelial neoplasia (CIN). Sustained E7 protein expression is the pathological basis for CIN and cervical cancer. We collected the cervical cell samples of women who visited the gynecological clinic of Peking Union Medical College Hospital between September 2018 and September 2019 and submitted them to the high-risk human papillomavirus (Hr-HPV) test. We performed a magnetic particle-based chemiluminescence enzyme immunoassay to analyze the HPV16/18 E7 protein level in CIN of different severities and compared the results with those of cervical pathology (gold standard) and the HPV test. The positive rate of HPV16/18 E7 protein increased with the severity of CIN: 26.6% in normal tissue, 58.3% in CIN1, and 70.6% in CIN2 or higher (CIN2+). For CIN2+, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the E7 protein were 70.6, 67.9, 52.2, and 82.3%, respectively. These values of the HPV test were 86.8, 44.5, 43.7, and 87.1%, respectively. With the combination of the E7 protein assay and HPV test, the specificity for diagnosing CIN2+ was 78.1%, which was significantly higher than that of the HPV test alone. HPV16/18 E7 protein level is correlated with the severity of CIN and has a high concordance rate with the pathological result. For cervical cancer screening, the combination of HPV16/18 E7 protein assay and HPV test improves the CIN diagnostic specificity, detection rate, and detection accuracy.

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| *Wu et al.* | *BMC Med.* | *2021* | *Development of models for cervical cancer screening: construction in a cross-sectional population and validation in two screening cohorts in China* |

**Summary:**
Current methods for cervical cancer screening result in an increased number of referrals and unnecessary diagnostic procedures. This study aimed to develop and evaluate a more accurate model for cervical cancer screening. Multiple predictors including age, cytology, high-risk human papillomavirus (hrHPV) DNA/mRNA, E6 oncoprotein, HPV genotyping, and p16/Ki-67 were used for model construction in a cross-sectional population including women with normal cervix (N = 1085), cervical intraepithelial neoplasia (CIN, N = 279), and cervical cancer (N = 551) to predict CIN2+ or

CIN3+. A base model using age, cytology, and hrHPV was calculated, and extended versions with additional biomarkers were considered. External validations in two screening cohorts with 3-year follow-up were further conducted (NCohort-I = 3179, NCohort-II = 3082). The base model increased the area under the curve (AUC, 0.91, 95% confidence interval [CI] = 0.88–0.93) and reduced colposcopy referral rates (42.76%, 95% CI = 38.67–46.92) compared to hrHPV and cytology co-testing in the cross-sectional population (AUC 0.80, 95% CI = 0.79–0.82, referrals rates 61.62, 95% CI = 59.4–63.8) to predict CIN2+. The AUC further improved when HPV genotyping and/or E6 oncoprotein were included in the base model. External validation in two screening cohorts further demonstrated that our models had better clinical performances than routine screening methods, yielded AUCs of 0.92 (95% CI = 0.91–0.93) and 0.94 (95% CI = 0.91–0.97) to predict CIN2+ and referrals rates of 17.55% (95% CI = 16.24–18.92) and 7.40% (95% CI = 6.50–8.38) in screening cohort I and II, respectively. Similar results were observed for CIN3+ prediction. Compared to routine screening methods, our model using current cervical screening indicators can improve the clinical performance and reduce referral rates.

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| *Torres-Ibarra et al.* | *Int J Cancer.* | *2021* | *Adjunctive testing by cytology, p16/Ki-67 dual-stained cytology or HPV16/18 E6 oncoprotein for the management of HPV16/18 screen-positive women* |

**Summary:**

High-risk human papillomavirus type 16/18 (HPV16/18) genotyping is unable to accurately discriminate nonprogressive infections from those that will progress to cervical cancer. Our study aimed to assesses if additional testing either with liquid-based cytology (LBC) or the putative progression markers p16/Ki-67 and HPV16/18 E6 oncoprotein (E6) can improve the efficiency of HPV16/18 genotyping for triaging high-risk HPV (hrHPV)-positive women through better cancer risk stratification. Women attending colposcopy after positive HPV16/18 genotyping results within the Forwarding Research for Improved Detection and Access for Cervical Cancer Screening and Triage (FRIDA) hrHPV-based screening study in Tlaxcala, Mexico, underwent further testing with LBC, p16/Ki-67 dual-stained (DS) cytology and E6. We calculated measures of test performance for detecting histologically confirmed cervical intraepithelial neoplasia grade 2 or higher (CIN2+) and grade 3 or higher (CIN3+). A number of 475 (64.3%) of 739 HPV16/18-positive women had complete results for all tests. Triage positivity rates were 14.1%, 18.5% and 24.4%, for LBC, E6 and DS, respectively. Compared with LBC, DS had higher sensitivity (24.4% vs 60.0%) although lower specificity (87.0% vs 79.3%) for CIN3+ ($P < .001$), whereas E6 had a sensitivity of 37.8% and a specificity of 83.5%. No invasive cancer was missed by DS or E6, but 75% were in normal cytology. DS test was associated with nearly 75% reduction of colposcopy referrals compared with the direct referral of all HPV16/18-positive women, giving the least number of colposcopies (n = 4.3) per CIN3+ detected. We show that adjunctive testing of HPV16/18-positive women with DS may greatly reduce unnecessary colposcopy referrals within HPV-based screening employing HPV16/18 genotyping while retaining acceptable sensitivity for CIN2+ and CIN3+.

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| *Bhattarakosol et al.* | *J Virol Methods.* | *2018* | *Immunogold-agglutination assay for direct detection of HPV-16 E6 and L1 proteins from clinical specimens* |

**Summary:**

HPV-16 infection is the most common cause of cervical cancer. As HPV-16 transforms the cell, E6 oncoprotein is over-expressed. Therefore, molecular detection of HPV-16 E6 mRNA is now being used for diagnosis and prediction of cancer development. Besides detecting E6 mRNA, a rapid lateral flow detecting the E6 protein using enzyme immunoassay is also now on market with a sensitivity of 53.5% for cervical intraepithelial neoplasia (CIN)-3 or more severe (CIN-3+). Here, an immunogold-agglutination assay was developed to detect not only HPV-16 E6 protein but also L1, a major capsid protein found in the productive stage of the virus. Evaluation of this test using HPV-16 DNA positive cervical samples showed that the HPV-16 E6 immunogold-agglutination assay results correlated well with the progression of the cervical lesions, i.e., 10.34% of CIN-1, 68.75% of CIN-3 and 80% of cancer (CaCx) and none for healthy normal samples. Interestingly, the HPV-16 L1 protein was found in most of the cases with cancer indicating the possibility of virion production. Immunogold-agglutination assay for E6 protein is simpler, easier to be performed with a sensitivity of 73.1% for CIN-3+ suggesting a good method for laboratory diagnostic use.

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| *Ndizeye et al.* | *BMJ Open.* | *2019* | *Performance of OncoE6(TM) Cervical Test in detecting cervical precancer lesions in HIV-positive women attending an HIV clinic in Bujumbura, Burundi: a cross-sectional study* |

**Summary:**
New rapid and low-cost molecular tests for cervical cancer screening, such as the OncoE6 Cervical Test, are emerging and could be alternatives for low-income and middle-income countries. To this end, we evaluated the clinical performance of the OncoE6 Cervical Test in detecting cervical intraepithelial neoplasia (CIN) among HIV-infected women in Bujumbura, Burundi. From June to December 2017, a cross-sectional study was conducted in 680 HIV-positive women at the University Hospital. Women aged 25-65 years who declared having had vaginal intercourse were consecutively recruited, and cervical specimens for OncoE6, liquid-based cytology and human papillomavirus (HPV) genotyping were obtained and visual inspection with acetic acid performed. Thereafter, participants underwent a colposcopic examination. The sensitivity, specificity, and positive and negative predictive values of the different tests were calculated with reference to 'colposcopic-histological' diagnoses, and areas under the receiver operating curves of OncoE6 and cytology tests were compared. The prevalence of CIN was 4.9%, and OncoE6 positivity was 3.1%. OncoE6 sensitivity varied from poor to low with increasing disease severity (42.1%, 95% CI 19.9% to 64.3% at CIN2+ threshold; and 58.3%, 95% CI 30.4% to 86.2% at CIN3+ threshold). OncoE6 had the highest specificity compared with all other tests used together. The performance of the OncoE6 test was significantly lower compared with cytology at atypical squamous cell of undetermined significance (ASCUS+) cut-off (AUC=0.68 vs 0.85, p=0.03) and low-grade squamous intraepithelial lesion (LSIL+) cut-off (AUC=0.68 vs 0.83, p=0.04) for CIN2+ diagnoses. However, the performance of the OncoE6 test was similar to that of cytology at high-grade squamous intraepithelial lesion (HSIL+) cut-off (AUC=0.68 vs 0.76; p=0.30) for CIN2+ diagnoses and was also similar to that of cytology at all cut-offs (ASCUS+, LSIL+ and HSIL+) for CIN3+ diagnoses (p1=0.76, p2=0.95 and p3=0.50, respectively). The current OncoE6 test proved to be a point-of-care test. However, given its poor performance for CIN2+ diagnoses, we do not recommend it for primary screening. We recommend to enrich it with more oncogenic HPV types, which may improve the performance of the test akin to that of cytology.

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| *Zhao et al.* | *Cancer Prev Res* | *2013* | *An Evaluation of Novel, Lower-Cost Molecular Screening Tests for Human Papillomavirus in Rural China* |

**Summary:**
New, lower-cost tests that target high-risk human papillomavirus (HR-HPV) have been developed for cervical cancer screening in lower-resource settings but large, population-based screening studies are lacking. Women ages 25 to 65 years and living in rural China (n = 7,543) self-collected a cervicovaginal specimen, had 2 cervical specimens collected by a clinician, and underwent visual inspection after acetic acid (VIA). The self- and one clinician-collected specimens underwent HR-HPV DNA testing by careHPV (QIAGEN) and Hybrid Capture 2 (HC2; QIAGEN) and the other clinician-collected specimen was tested for HPV16, 18, and 45 E6 using OncoE6 (Arbor Vita Corporation). Women who screened positive for any test and a random sample of those negative on all tests underwent colposcopic evaluation. The percent test positive was 1.8% for HPV E6 oncoprotein, between 14% and 18% for HR-HPV DNA testing, and 7.3% for VIA. The sensitivity for cervical intraepithelial neoplasia grade 3 or more severe (CIN3(+); n = 99) was 53.5% for OncoE6, 97.0% for both careHPV and HC2 testing of the clinician-collected specimen, 83.8% for careHPV testing and 90.9% for HC2 testing of the self-collected specimen, and 50.5% for VIA. OncoE6 had the greatest positive predictive value (PPV), at 40.8% for CIN3(+), compared with the other tests, which had a PPV of less than 10%. OncoE6 tested 70.3% positive for HPV16, 18, or 45-positive CIN3(+) and tested negative for all HPV16-, 18-, or 45-negative CIN3(+) ($P < 0.0001$). HPV E6 oncoprotein detection is useful for identifying women who have cervical precancer and cancer.

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| *Schweizer et al.* | *J Clin Microbiol.* | *2010* | *Feasibility Study of a Human Papillomavirus E6 Oncoprotein Test for Diagnosis of Cervical Precancer and Cancer* |

**Summary:**
In a feasibility study using a prototype, lateral-flow test for human papillomavirus type 16, 18, and/or 45 (HPV16/18/45) E6 oncoproteins, 51 of 75 (68%; 95% confidence interval [95% CI] of 56 to 78%) of HPV16/18/45 DNA-positive specimens from women with a diagnosis of CIN3+ (cervical intraepithelial neoplasia grade 3+ or cervical cancer) tested positive for HPV16/18/45 E6 oncoprotein. None of 16 (95% CI of 0 to 37%) HPV16/18/45 DNA-positive cervical specimens from women with a negative or CIN1 diagnosis tested positive for HPV16/18/45 E6 oncoprotein.

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| *Valdez et al.* | *Int J Cancer.* | *2015* | *Effectiveness of novel, lower cost molecular human papillomavirus-based tests for cervical cancer screening in rural china transmitted infections in low- and middle-income countries* |

**Summary:**
This study examined the efficacy of the OncoE6™ Cervical Test, careHPV™ and visual inspection with acetic acid (VIA) in identifying women at risk for cervical cancer and their capability to detect incident cervical precancer and cancer at 1-year follow-up. In a population of 7,543 women living in rural China, women provided a self-collected and two clinician-collected specimens and underwent VIA. All screen positive women for any of the tests, a ~ 10% random sample of test-negative women that underwent colposcopy at baseline, and an additional ~ 10% random sample of test-negative women who did not undergo colposcopy at baseline (n = 3,290) were recruited. 2,904 women were rescreened 1 year later using the same tests, colposcopic referral criteria, and procedures. Sensitivities of baseline tests to detect 1-year cumulative cervical intraepithelial neoplasia Grade 3 or cancer (CIN3+) were 96.5% and 81.6% for careHPV™ on clinician-collected and self-collected specimens, respectively, and 54.4% for OncoE6™ test. The OncoE6™ test was very specific (99.1%) and had the greatest positive predictive value (PPV; 47.7%) for CIN3+. Baseline and 1-year follow-up cervical specimens testing HPV DNA positive was sensitive (88.0%) but poorly predictive (5.5-6.0%) of incident CIN2+, whereas testing repeat HPV16, 18 and 45 E6 positive identified only 24.0% of incident CIN2+ but had a predictive value of 33.3%. This study highlights the different utility of HPV DNA and E6 tests, the former as a screening and the latter as a diagnostic test, for detection of cervical precancer and cancer.