

Diagnostic Accuracy of the Rapid Human Papillomavirus Oncoprotein E6/E7 Antigen Test for Cervical Cancer Screening in a Low-Resource Setting: A Cross-Sectional Study

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Abstract

Background: Persistent high-risk human papillomavirus is responsible for 95% of all cases of cervical cancer worldwide. Screening for high-risk genotypes has been acknowledged as the gold standard for the elimination of cervical cancer. However, most low- and middle-income countries have limited access to this screening method because of a lack of adequate sustainable resources.

Objective: This study aimed to compare the diagnostic accuracy of the rapid high-risk human papillomavirus 16/18 oncoprotein E6/E7 antigen test to that of the polymerase chain reaction (PCR) deoxyribonucleic acid test among women who presented to family planning and dysplasia clinics at Moi Teaching and Referral Hospital.

Methods: This was a cross-sectional study performed between February and July 2024 to compare the diagnostic accuracy of the rapid high-risk human papillomavirus 16/18 oncoprotein E6/E7 antigen test to that of the polymerase chain reaction deoxyribonucleic acid test among women who underwent cervical cancer screening at Moi Teaching and Referral Hospital. Two hundred and fifty-seven (257) women aged 30 years and older were randomly selected for this study. A cervical swab was obtained from all participants for human papillomavirus polymerase chain reaction deoxyribonucleic acid testing, followed by cervical sample collection using the Delphi vaginal self-sampler for the rapid human papillomavirus oncoprotein E6/E7 antigen test. To achieve the objective of the study, sensitivity, specificity, positive predictive value, and negative predictive value were measured. Data on sociodemographic and clinical characteristics were collected and analysed. The significance of the data was set at a two-tailed $p < 0.05$. The chi-square test was used to compare categorical variables. Fisher's exact test or the Monte Carlo correction was used for the chi-square analysis when more than 20% of the cells had an expected count < 5 .

Results: The sensitivity of the rapid oncoprotein E6 and E7 test was 99.33%, and the specificity was 99.07%. In addition, the positive predictive value and negative predictive value were 99.33% and 99.07%, respectively. Furthermore, the accuracy of the rapid oncoprotein E6/E7 test was 99.22%. These findings were statistically significant ($p < 0.001$, 95% CI=0.98–1.00), and the AUC for the rapid oncoprotein E6/E7 test was 0.992.

Conclusion: The accuracy of the rapid high-risk human papillomavirus mRNA oncoprotein E6/E7 antigen test is as good as that of the polymerase chain reaction deoxyribonucleic acid test for cervical cancer screening in low-resource settings.

Introduction

Persistent high-risk human papillomavirus (HR-HPV) is responsible for 95% of all cases of cervical cancer worldwide [1]. In low- and middle-income countries, the prevalence of HR-HPV and the incidence of cervical cancer remain high due to limited access to preventive measures and treatment [2]. Most of these countries lack acceptance of primary prevention measures and lack access to accurate and cost-

effective screening methods [3]. The cervical HPV test, especially the polymerase chain reaction deoxyribonucleic acid (PCR DNA) test, has been acknowledged as the standard cervical cancer screening modality because of its high sensitivity and specificity. This test has offered a great opportunity to improve the effectiveness of cervical cancer screening [3, 4, 7, 6]. The WHO global strategy has established three targets to be achieved by the year 2030 to position all countries on the pathway to eliminate this deadly and preventable disease [1]. However, sub-Saharan Africa (SSA), the cradle of HR-HPV and cervical cancer, has been struggling to achieve the World Health Organization's goal to eliminate cervical cancer through the prevention, screening, and treatment of premalignant cervical cancer lesions and early cervical cancer [1, 3].

To date, studies have shown that laboratory tests for the detection of high-risk HR-HPV are more sensitive and reliable than conventional cytology and visual inspection using acetic acid and/or Lugol iodine [5, 8, 9, 10, 11]. The currently available evidence has shown that both the sensitivity and specificity of the high-risk HPV DNA screening test are less for adenocarcinoma [12], but very good for squamous cell carcinoma, except [13]. Despite its acknowledgement as the gold standard screening method, its high cost, sophisticated equipment that requires additional training, the need for electricity, the need for regular maintenance of the equipment, expensive reagents, and long turnaround time of up to 2 weeks limit HPV DNA testing in low- and middle-income countries (LMICs) [13]. Economic assessments are relevant to support the decision to incorporate more cost-effective strategies for the screening and treatment of cervical cancer at LMICs [14]. The challenges in LMICs are numerous, and the lower socioeconomic status (SES) of most women in those countries unfortunately affects their health care-seeking behaviour, as poor health care literacy compromises their compliance with medical advice and their understanding of the diagnosis of their health problems. For example, a positive HR-HPV test may increase anxiety among women, although HPV infections are transient in most women. The current WHO guidelines for the screening of cervical cancer in LMICs are challenged by limited resources, and thus follow-up after positive results are obtained at first screening can be difficult [14].

According to the WHO, several HPV tests are available: some HPV tests detect DNA, while other HPV tests detect the oncoprotein E6/E7 mRNA [15]. Tests that are currently commercially available and that are being used in some countries for cervical cancer screening include the Hybrid Capture 2 (Qiagen), CareHPV (Qiagen), Cobas HPV Test (Roche), Cervista (Hologic), Aptima HPV Assay (Hologic), BD HPV Assay (BD) and Xpert HPV (Cepheid) [15, 16]. In selecting which HPV test will be used in a given screening program, consideration should be given to the results of clinical trials, clinical validation of the test, and other operational and logistical aspects of the test and its requirements [16].

The carcinogenesis of the cervix is mediated by the HPV-E6 and E7 oncoproteins, which are biomarkers that are useful for managing women with positive screening results. To date, evidence has established that these 2 oncoproteins play a major role in promoting and maintaining cervical carcinogenesis, as they are overexpressed during cervical transformation [14, 17]. The oncoprotein E6 plays a major role in cell immortalization, as it binds to the tumour suppressor protein p53, which is usually activated in normal cells in response to DNA damage. This binding degrades p53 and prevents the cell from

undergoing apoptosis [17]. When the oncoprotein E7 interacts with host proteins, it binds to the cellular tumour suppressor pRb, which leads to its degradation, preventing it from inactivating transcription factors and promoting cell proliferation [18]. In addition, the overexpression of E6 and E7 leads to the accumulation of genetic errors in cells over time, which when combined with viral gene deregulation, drives invasive cancer [19, 20]. Thus, oncoproteins E6 and E7 are interesting markers of persistent HR-HPV infection and associated precancerous lesions.

Readers may ask what the difference is between the test for the E6 and E7 oncoproteins and the HPV DNA genotype test in cervical cancer screening. The oncoproteins E6 and E7 differ according to HPV genotype. Thus, tests must target oncoproteins from diverse oncogenic types to ensure high sensitivity and specificity [21]. The clinical accuracy of E6 or E7 DNA and RNA assays has been demonstrated in several studies [14, 16, 21]. However, few studies in sub-Saharan Africa have been conducted to determine the accuracy of rapid HR-HPV mRNA oncoprotein E6/E7 detection. To fill this gap, this study aimed to determine the diagnostic accuracy of the HR-HPV mRNA oncoprotein E6/E7 test in a low-resource setting as a cost-effective cervical cancer screening modality.

Materials and methods

Study design, setting, and population

This was a cross-sectional study performed at Moi Teaching and Referral Hospital, the second-largest national hospital in Kenya. This multispecialty hospital, which serves a population of over 25 million people in Kenya, parts of Eastern Uganda, South Sudan, Northern Tanzania, and the Democratic Republic of the Congo, is known for its high-quality health care. In partnership with the Academic Model Providing Access to Health care (AMPATH), the hospital offers screening, prevention, and treatment for gynaecological cancers. Since screening for the cervical program started in 2009, hospitals and their satellites have essentially used visual inspection with acetic acid, and occasionally, Pap cytology and HPV PCR DNA testing. The government of Kenya approved and adopted the HPV PCR DNA test as the gold standard screening method for cervical cancer in 2018; however, the high cost of the test and other constraints previously mentioned have limited its implementation in rural areas.

In November 2023, a feasibility study was conducted using rapid testing of HPV oncoproteins E6 and E7, Pap cytology, and PCR. This feasibility study enrolled 185 participants. Both samples collected by the patients and those collected by clinicians were used for rapid testing of HPV oncoproteins E6 and E7. For the current study, 257 participants were included. All women aged between 30 and 65 years who attended the family planning and dysplasia clinics at Moi Teaching and Referral Hospital within the study period and who provided informed consent to participate in this study were included. Considering the short period of data collection, pregnant women, women aged less than 30 years, and women with confirmed diagnoses of cervical cancer were excluded. Those who were menstruating were temporarily excluded until bleeding ceased. All eligible women were approached and given information about cervical cancer screening and the study, methods of sample collection, the two HR-HPV tests, and the

meaning of the results (negative and positive) before providing informed consent. Figure 1 illustrates the recruitment process for the participants.

The data collection was conducted by experienced and trained research assistants, including 2 registered nurses (RNs), 3 medical doctors, and 1 social worker. Written informed consent was obtained from each participant. Women who could not read or write were assisted by a social worker in the presence of either a relative or a social worker.

Questionnaire survey

The research assistants (nurses and doctors) administered a questionnaire that was developed in English and translated into Kiswahili. The data included sociodemographic and clinical characteristics. The research assistants provided counselling to each study participant and explained the implications of an HR-HPV-positive result. Three to four weeks after the HPV sampling, the participants received results either through a message (those with negative results) or in person (those with positive results).

Sample collection for detection of the HPV oncoproteins E6 and E7

The eligible women were assisted by either the RN or the medical doctor during sample collection using the Delphi vaginal self-sampler (DVSS). All research assistants were trained for 3 months before the start of the study, and then, refresher training was performed 3 days before the enrolment of participants. The kits did not contain expired materials, and precautions were taken to avoid cross-contamination of the samples; a new specimen collection container was used for each sample. After the patient was placed on the gynaecological examination table and the speculum was inserted, the excess mucus discharge was removed from the cervix with a cotton ball and discarded. The DVSS was inserted, and the sample was collected according to the manufacturer's instructions. This process was performed for each participant. The collected sample was immediately prepared for testing as described below.

HPV DNA PCR sample collection

The eligible women were assisted by either the RN or the medical doctor when HPV DNA was collected using an Evalyn Brush[®] (Evalyn Rovers Medical Devices BV, the Netherlands) for HR–HPV DNA tests. The Evalyn[®] Brush is validated for use in PCR-based assays, which ensures the highest number of valid samples across all age groups. In addition, the use of a dry sample (with no liquid in the mail) makes it compatible with the Cobas[®] 6800 or 4800 system for polymerase chain reaction (PCR). The collected HR-HPV DNA samples were transported daily to the Lancet-Kenya laboratory, Eldoret branch, and were stored in the Department of Pathology at room temperature (approximately 19–24 °C) for a maximum of 2 weeks.

HPV oncoprotein E6 and E7 specimen testing

The collected sample was prepared using a pipette (dropper). Eight (8) drops were extracted from the tube for each sample. Eight (8) drops of extraction buffer A were added to the extracted sample, and the

mixture was shaken well. After 2 minutes, 8 drops of extraction buffer B were added to the sample, which was incubated with the buffer for 1 minute. Three drops (approximately 100 µl) were extracted from the tube to the sample well onto the cassette. For each prepared sample, the result was read within 15 minutes following extraction from the tube to the sample well onto the cassette. The result was positive when 2 coloured (red-like) bands appeared on the membrane (cassette): one band appeared in the control region (C), and another band appeared in the test region (T). In contrast, the result was negative when only one coloured band appeared in the control region, while the band in the test region (T) was not coloured. The appearance of a faint coloured band in the control region was reported as positive.

The rapid test for HPV oncoproteins E6 and E7 is manufactured for the qualitative detection of the HR-HPV 16/18/13/45/52/58 E6 and E7 oncoproteins only, but without segregation, as in the HPV DNA PCR test.

HPV DNA specimen testing

The samples collected were also transferred daily to Lancet Kenya, Eldoret branch, for testing. The samples were processed biweekly using the Cobas[®] 6800 system for HPV DNA polymerase chain reaction (PCR). This system detects the presence of 14 HR-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68). The results are automatically interpreted by the HPV DNA PCR test system and are displayed graphically on a controller monitor screen as either 'positive' or 'negative for a specific HR-HPV type [22, 23, 24, 25]. The samples were analysed by trained laboratory technicians from a certified laboratory, Cerba Lancet Kenya. This study did not receive ethics committee approval for a Pap test, which could have been impactful and useful.

Clinical relevance of this study

This study helps determine the clinical impact of using the rapid HR-HPV oncoprotein E6/E7 test, which is an affordable and accessible cervical cancer screening modality, and helps guide decision-making about whether to incorporate this test into routine practice in low-resource settings.

Management of the results and future plans for participants

The precaution of releasing positive results in a doctor's office was adopted to reduce anxiety associated with such results. Appropriate counselling was provided to emphasize that HPV-positive results from the rapid HPV oncoprotein E6 and E7 test require confirmatory tests with HR-HPV PCR DNA. In addition, HR-HPV DNA PCR-positive results need to be paired with the results of another test—cytology or with—and patients should be referred for colposcopy and follow-up care [26, 27].

Statistical analyses

The statistical analyses were conducted using the statistical software package SPSS V.20.0 (Armonk, NY: IBM Corp.). Qualitative data are presented as numbers and percentages. The Kolmogorov–Smirnov

test was used to verify the normality of the distribution. The quantitative data are presented as ranges (minimums and maximums), means, standard deviations, medians, and interquartile ranges (IQRs).

Statistical analysis of the accuracy of the rapid HPV oncoprotein E6/E7 test quantified how well the test correctly identified individuals with or without a disease according to measures such as sensitivity, specificity, and predictive values. The rapid test reported the results as either positive or negative, whereas the HPV PCR DNA test quantitatively separated the results into 3 groups: HR-HPV 16, HR-HPV 18, and HR-HPV non-16/18. On the basis of the interpretation of the PCR DNA results, these results were reported as either positive or negative for the specific genotype mentioned above. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for the detection of HR-HPV in each test were calculated. A positive result was defined when both tests were positive or when either test was negative. The sensitivity of the tests refers to the proportion of individuals with human papillomavirus infection who were correctly identified by the test (true positives), whereas the specificity of the tests refers to the proportion of individuals without the disease who were correctly identified by the test (true negatives) [28]. The results of the two diagnostic tests were interpreted to determine whether the participants had or did not have high-risk human papillomavirus infection according to the predictive values (positive or negative). The probability that participants with positive test results were actually infected with HR-HPV was referred to as the positive predictive value, whereas the probability that those with negative results were actually negative was referred to as a negative predictive value [29].

Furthermore, the overall diagnostic ability of the test to provide an accurate diagnosis was assessed using the area under the receiver operating characteristic (ROC) curve (AUC) [30, 31]. The ROC curve results graphically displayed the effective sensitivity for each specificity for high-risk human papillomavirus infection. The significance of the results was judged at the 5% level.

Results

Sociodemographic characteristics

The mean age of the participants was 40.8 ± 9.1 years, as described in Table 1. Women aged between 40 and 50 years accounted for 38.1% of all patients, while those aged between 30 and < 40 years accounted for 45.5%. With respect to the marital status of the participants, most were married women and widows and represented 58.4% and 17.9% of all patients, respectively. Participants with primary and secondary education levels were almost equally represented. More than half of the participants were unemployed (see Table 1).

HPV status of the participants

With respect to the HPV status of the participants, 58.0% reported positive results for rapid tests that used mRNA, whereas 42% of the participants tested negative. For the PCR test, 7.4% of the participants tested positive for high-risk HPV 16, whereas 3.1% tested positive for high-risk HPV 18. In addition,

33.5% of the participants tested positive for non-high-risk HPV 16/18 (see Table 2). Table 3 shows the concordance between the rapid oncoprotein E6/E7 and PCR DNA tests in 77% of the cases.

Accuracy of rapid HR-HPV oncoprotein detection

Table 4 shows that the sensitivity of the rapid oncoprotein E6 and E7 test was 99.33% and that the specificity was 99.07%. In addition, the positive and negative predictive values were 99.33% and 99.07%, respectively. The accuracy of the rapid oncoprotein E6 and E7 test was 99.22%. These findings were statistically significant ($p < 0.001$, 95% CI=0.98–1.00), and the AUC for the rapid oncoprotein E6 and E7 test was 0.992.

The accuracy of the HR-HPV PCR DNA test was individually assessed according to the human papillomavirus genotypes, as summarized in Table 4 and graphically demonstrated in Figure 2. The sensitivity and specificity for HR-HPV 16 were 89.26% and 100.0%, respectively. In addition, the positive and negative predictive values for the HR-HPV 16 PCR DNA test were 100.0% and 87.10%, respectively. The accuracy of the HR-HPV 16 PCR DNA test was 93.77%, with an AUC of 0.946. This difference was statistically significant ($p < 0.001$, 95% CI=0.92–0.98). For HR-HPV 18, the sensitivity and specificity were 95.30% and 100.0%, respectively. The positive and negative predictive values were 100.0% and 93.91%, respectively, with an estimated diagnostic accuracy of 97.28% and an AUC of 0.977. This difference was statistically significant ($p < 0.001$, 95% CI=0.96–0.99). With respect to the PCR DNA test for HR-HPV non-16/18, the sensitivity and specificity of the test were 39.60% and 100.0%, respectively, whereas the positive and negative predictive values were 100.0% and 54.55%, respectively. The accuracy of the PCR DNA test for HR-HPV non-16/18 was 64.98%, with an AUC of 0.698. This difference was statistically significant ($p < 0.001$, 95% CI=0.64–0.76).

Discussion

The overall positive rate of the HR-HPV oncoproteins E6/E7 observed in the present study was lower (58.0% vs. 70%) than that reported in Brazil by Valença, J. E. C et al. (2016) [32] and the 100% positive rate reported in a Korean study [33]. In contrast with the current study, both the Brazilian and Korean studies were conducted on patients who were diagnosed with low-grade and high-grade squamous intraepithelial lesions. The three studies used HR-HPV tests targeting the E6 and E7 mRNAs using different kits with novel technologies. The current study used qualitative E6 and E7 tests, whereas the 2 previous studies used quantitative E6 and E7 mRNA tests [32, 33] to identify the specific HR-HPV genotype. Although the qualitative E6 and E7 mRNA tests used could not specify the HR-HPV genotype, the tests were able to report positivity for the E6/E7 oncoproteins, while the PCR DNA test separated the results into the HPV 16, HPV 18, or non-HPV 16/18 groups. The low positivity rates reported in this study may be related to the grade of the cervical lesions (low grade), which is currently difficult to determine. Follow-up care with colposcopy or cytology and biopsy for those with positive test results is important. However, previous studies have shown that the mRNA positivity rate is increased in patients with more severe cervical intraepithelial lesions [34, 35].

The sensitivity of the oncoprotein E6/E7 mRNA reported in this study was greater than that reported by Benevolo, M., and colleagues (2011) [34], and recently, that by Downham, L., et al. (2024) [36]. In contrast to the current study findings, the other 2 studies were conducted under similar circumstances in patients who were diagnosed with cervical intraepithelial neoplasia (CIN) or abnormal cytology. In the first study, the sensitivity varied according to the severity of CIN. For example, Benevolo M. et al. reported an HPV mRNA sensitivity of 83% in atypical squamous cells of undetermined significance (ASCUS), 62% in low-grade squamous intraepithelial lesions (LSILs), and 67% in high-grade squamous intraepithelial lesions (HSILs) [34]. However, the corresponding sensitivities in the same study were 99%, 91%, and 96% for the HR-HPV DNA PCR test [34]. In one recent systematic review, the pooled sensitivity of an enzyme-linked immunosorbent assay (ELISA) (100% vs. 18%) was reported, whereas the pooled sensitivity of a multiplex HPV serology assay was reported to be 16% [37], which is lower than the sensitivity reported for the rapid detection of HPV mRNA. As in the APTIMA mRNA study, the higher sensitivity compared with that in previous studies may be because the samples were collected by clinicians versus the patients.

In the present study, the sensitivity of the HR-HPV PCR DNA test was low for the detection of HR-HPV 16 and 18 and extremely low for HR-HPV non-16/18 compared with the sensitivity of the rapid oncoprotein E6/E7 test. This is difficult to explain because the sensitivity of the diagnostic test is not influenced by the prevalence of the disease [30, 31, 32, 33]. In addition, PreTect HPV-Proofer was used to detect the mRNAs of the HPV oncoproteins E6 and E7, whereas the current study used the rapid HPV oncoprotein E6 and E7 mRNA test method. In the Aptima study [38], the sensitivity was 96.3%, which was similar to what was reported in the current study. These findings indicate that mRNA oncoprotein tests are good cervical cancer screening modalities, especially in low- and middle-income countries.

In this study, the specificity of the current rapid E6/E7 oncoprotein test was greater than that reported by Benevolo M. et al. [34]. In the study by Benevolo and colleagues, low specificity was observed, especially for ASCUS and LSIL (63.2% vs. 82 and 76), while higher specificity was found for HSIL [34]. The Pap test was not approved by the ethics committee for the current study, and thus any data comparison is difficult. One systematic review revealed that HPV16 and HPV18 oncoprotein E6 and E7 antibodies have high specificity but low sensitivity for detecting cases of cervical cancer or CIN2/3 precancerous lesions [36]. In the Aptima study, the specificity of the LSIL was 43.2%, which was lower than the specificity reported in the current study. In contrast, the specificity of the HR-HPV DNA PCR test was optimally greater, which supports the superiority of tests excluding false positivity, whereas the Aptima study reported a specificity of 85%. Although the specificity of the HR-HPV DNA PCR test was 100% in the current study, several studies have reported low specificity of the HR-HPV DNA test and high specificity for HR-HPV mRNA tests (specificity as low as 29%, 13%, and 4% for ASCUS, LSIL, and HSIL, respectively) [2, 3, 39]. This is because high-risk HPV tests have low specificity in detecting high-grade cervical intraepithelial neoplasia (CIN2 or CIN3) [39, 40].

The positive predictive value (PPV) in the current study was comparable to that reported in another HPV mRNA study (99% vs. 96%) [41] and higher than that reported in yet another study, (80%) [31]. However,

the PPV reported in this previous study was exclusively for HSIL. The difference may be partially due to the different use purposes of HPV mRNA tests between the studies. The current study uses HPV mRNA tests as screening tests, whereas the other 2 studies used mRNA tests as triage tests to reduce the need for referrals for colposcopy. In the Ugandan study, the positive predictive value for the detection of any HR-HPV E6/E7 mRNA for the presence of CIN 2 + was 8.2% [42], which was lower than that reported with rapid HPV mRNA tests. In addition, the positive predictive value associated with the detection of HPV-16 mRNA was 15%, and that for the detection of HPV-18/45 mRNAs was 15% lower [43]. In contrast to the current hospital-based study, the Ugandan study was a community-based screening study. However, the positive predictive value of the HPV DNA PCR test was reported to be 100% for HR-HPV 16 and 18. This finding contrasts with what was observed by Aydogan Kirmizi, D. et al. (2021), who reported a PPV of 66.7% for LSIL and 70.0% for HSIL [41], which is similar to what was reported by Kaya Terzi, N. (2021) [44].

In this study, the negative predictive value was 100%, which is higher than the 91.9% recently reported by Kittisiam, T., et al. (2024) [45]. Few studies have been published on the negative predictive value of HPV DNA and mRNA.

The findings from the present study revealed that the accuracy of the rapid HPV mRNA test was greater than that of the HPV DNA test (99.07% vs. 87.10% for HPV16, 93.03% for HPV18, and 54.55% for HR-HPV non-16/18). These results are similar to observations made by the WHO through the generation data group (GDG) regarding the accuracy of HPV mRNA tests vs. HPV DNA tests [46, 47, 48]. In a recent systematic review conducted by Arbyn M. and colleagues [22], HPV mRNA tests, especially the APTIMA HPV assay, which targets the E6/E7 mRNA of HR-HPV, showed slightly lower pooled sensitivity but higher specificity than the standard comparator tests in seven screening studies [22]. In contrast to APTIMA, the rapid HPV assay targeting E6/E7 of HR-HPV16/18 showed high sensitivity and specificity as a promising primary screening test. The differences between the APTIMA and the rapid HR-HPV screening tests include the following: APTIMA is a quantitative test with a turnaround of 3–4 hours, which is similar to the GeneXpert HPV DNA tests, whereas the rapid HR-HPV 16/18 test is a qualitative test with a turnaround of 15 minutes.

The performance of the rapid oncoprotein E6/E7 test as a screening modality is similar to that of the PCR DNA test. Figure 2 shows this result better, as the AUC values of the tests were interpreted and compared with those of the HR-HPV genotypes. The ROC curve shows that the rapid oncoprotein E6/E7 test is highly accurate. Mander and colleagues reported that a diagnostic test is useful when its sensitivity and specificity are greater than 50% [30]. Therefore, the integration of rapid oncoprotein E6/E7 tests as a cervical cancer screening modality in LMICs will positively impact the strategy of eliminating cervical cancer.

Conclusions

The high sensitivity and relative specificity of the rapid HR-HPV E6 and E7 mRNA test support its use for primary cervical cancer screening. However, women with positive HR-HPV results should be referred for colposcopy and further follow-up care. Large-scale and longitudinal studies are needed to validate the rapid detection of E6/E7 oncoproteins as a primary cervical cancer screening method at the community level.

Strengths and limitations

- This study demonstrated that the rapid HPV oncoprotein test is as accurate as the PCR DNA test for cervical cancer screening.
- The negative predictive value observed is comparable to that of the PCR DNA test.
- The inclusion of faint results increases the number of false-positive results.
- Women with positive rapid HPV oncoprotein results should be referred for colposcopy.
- This study did not receive ethics committee approval for the Pap test to be performed on the study participants.

Abbreviations

ASCUS: Atypical squamous cells of undetermined significance

AUC: Area under the curve

CIN: Cervical intraepithelial neoplasia

DNA: Deoxyribonucleic acid

HPV: Human papillomavirus

HR: high-risk

HSIL: high-grade squamous intraepithelial lesions

LSIL: low-grade intraepithelial lesions

mRNA: messenger RNA

NPV: negative predictive value

PCR: Ribonucleic acid

PPV: positive predictive value

RNA: Ribonucleic acid

ROC: Receiver operating characteristic

Declarations

Human Ethics approval and consent to participate: This study received approval from the MTRH/MU-Institutional Research and Ethics Committee (IREC) under the approval number FAN: 0004670 and the National research permit number NACOSTI/P/24/33601.

Clinical Trial: Not applicable

Consent for publication: Consent was obtained during enrolment in the study.

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Competing interests: None to declare

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Authors' contribution contributors: All the authors reviewed the manuscript. Conceptualization, investigation, and methodology were performed by the TKC, PPA, and MEW. Project administration and resources were performed by (KM, CEK, MK, BRM, OBE, NJN, MB, KK, KWK, MRM, AWK). Supervision, data curation, and formal analysis were performed by PPA, IPM, TPK, and MEW. Validation, visualization, and rewriting of the manuscript were performed by all the authors (TKC, PPA, MEW, KM, CEK, MK, BRM, OBE, NJN, MB, KK, KWK, MRM, AWK, IPM, and TPK).

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48. AMERICA PI. INTEGRATING HPV TESTING IN CERVICAL CANCER SCREENING PROGRAMS

Tables

Table 1. Socio-demographic Characteristics (n=257)

Variables	No.	%
Age (years)		
30 – <40	117	45.5
40 – <50	98	38.1
≥50	42	16.3
Mean ± SD.	40.75 ± 9.10	
Median (Min. – Max.)	41.0 (30.0 – 69.0)	
IQR	32.0 – 47.0	
Marital Status		
Married	150	58.4
Single	39	15.2
Divorced	22	8.6
Widowed	46	17.9
Education level		
None/primary	107	41.6
Secondary	103	40.1
Tertiary	47	18.3
Occupation		
Employed	67	26.1
Self employed	61	23.7
Unemployed	129	50.2
Parity		
Nulliparous	97	37.7
Para 1 and above	160	62.3

IQR: Inter quartile range

SD: Standard deviation

Table 2. HPV Status of participants (n=257)

Discordance between PCR DNA and Rapid test	No.	%
Rapid test results		
Positive	149	58.0
Negative	108	42.0
PCR 16		
Positive	133	51.8
Negative	124	48.2
PCR 18		
Positive	142	55.3
Negative	115	44.7
NON-16/18		
Positive	59	23.0
Negative	198	77.0

Table 3. Discordance between rapid HPV test and PCR DNA (n=257)

PCR VS RAPID TEST	No.	%
Concordant	198	77.0
Discordant	59	23.0

Table 4. Accuracy of Rapid HR-HPV oncoprotein E6/E7 test compared to PCR DNA test for HR-HPV 16, 18, and NON-16/18

Tests	AUC	p	95% C.I	Sensitivity	Specificity	PPV	NPV	Accuracy
Rapid test results	0.992	<0.001*	0.98 – 1.00	99.33	99.07	99.33	99.07	99.22
PCR 16	0.946	<0.001*	0.92 – 0.98	89.26	100.0	100.0	87.10	93.77
PCR 18	0.977	<0.001*	0.96 – 0.99	95.30	100.0	100.0	93.91	97.28
PCR NON-16/18	0.698	<0.001*	0.64 – 0.76	39.60	100.0	100.0	54.55	64.98

AUC: Area Under a Curve

p-value: Probability value

CI: Confidence Intervals

NPV: Negative predictive value

PPV: Positive predictive value

Figures



Figure 1

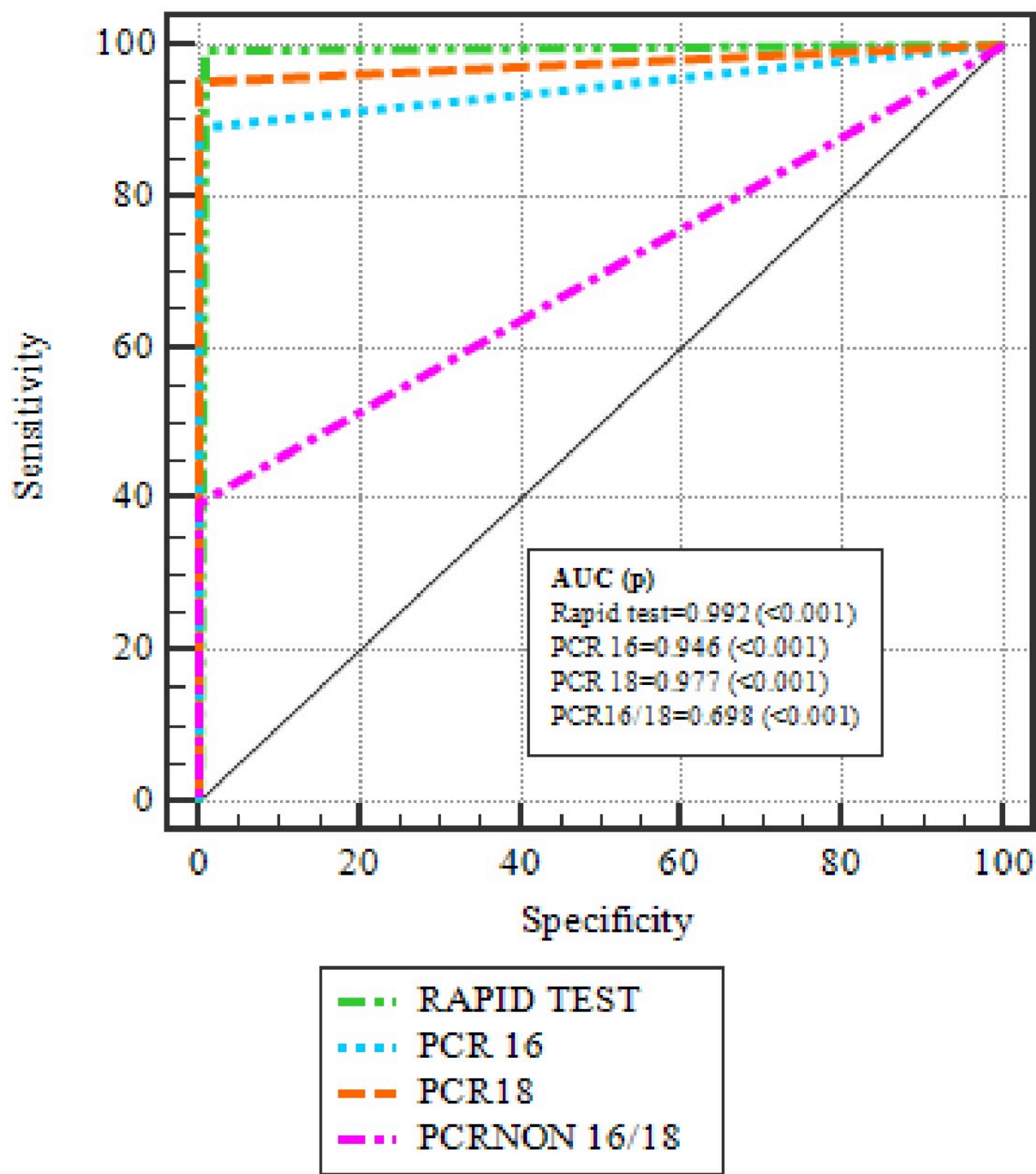


Figure 2

Sensitivity and specificity between rapid HR-HPV oncoprotein mRNA and PCR DNA tests