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Review Article

Point-of-care tests for human papillomavirus detection in uterine cervical samples: A review of advances in resource-constrained settings

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Incidence of cervical cancer and associated mortality are still high in resource-constrained countries due to the lack of infrastructural facilities and trained workforce. Human papillomavirus (HPV)-based screening tests offer a better sensitivity (>90%) for the detection of cervical high-grade lesions. However, these tests usually require an extensive laboratory set-up and trained technical staff. Moreover, the high cost of the currently available and approved HPV tests precludes their use in the cervical cancer screening programmes in resource-limited settings. Hence, there is a felt need for a low-cost point-of-care (POC) HPV test with good performance characteristics to help augment cervical cancer screening in such settings. A recent meta-analysis demonstrated a good sensitivity and specificity for two of the commercially available POC HPV tests. The present review discusses the merits and limitations of the current commercially available POC and near-POC devices for HPV-based cervical cancer screening. The technologies that have the potential to be developed into low-cost POC tests and newer promising modalities for HPV-based POC or near POC have also been highlighted. This review underscores the need for collaborative and coordinated research for development of POC or near-POC HPV-based tests to be used in cervical cancer screening. Efforts need to be focussed on technologies that offer ease of performance without the requirement of sophisticated equipment or extensive sample pre-processing coupled with a good sensitivity and cost-effectiveness.

Key words Cervical cancer screening - HPV DNA detection - near point-of-care - point-of-care

Human papillomavirus (HPV) is the causative agent of cervical precancerous lesions and cancer[1]. Currently, there are six US-FDA-approved assays for HPV detection (DNA or RNA) for use in cervical cancer screening[2]. In addition, the International HPV Laboratory Network conducts proficiency testing for laboratories using commercially available HPV-based tests as well as the in-house assays[3]. However, the cost-intensiveness of these assays, the requirement of an extensive laboratory set-up with high-end equipment and the delay in report generation (usually due to the batch system of sample processing) have hindered the utility and application of HPV-based cervical cancer screening in the low- and low-middle income countries[4]. Hence, there has been a felt need for the development of cost-effective point-of-care (POC) or near-POC methods for HPV detection in cervical samples. Alongside this, there has been a growing

interest in the evaluation of the self-sampling of vaginal samples for HPV-based cervical cancer screening to expand the coverage of screening. Studies have reported a comparable sensitivity across self-collected vaginal samples as well as physician-collected samples for the detection of cervical precancerous lesions[5,6]. The coupling of low-cost POC or near-POC HPV tests with self-sampling can offer the best opportunity to resource-constrained nations for cervical cancer elimination. However, a few studies have reported a lack of awareness (of cervical cancer as well as the importance of screening), social influence, religious reasons and partner's disapproval as barriers for women not consenting to participate in self-sampling for the HPV-based cervical cancer screening[7].

Over the last two decades, only a few studies have evaluated a POC test for HPV detection or described a technique that could potentially be utilized for a POC test. A recently published systematic review and meta-analysis[8] evaluated the performance of two POC tests for HPV detection, careHPV™ and OncoE6 cervical test™. This analysis[8] demonstrated a higher sensitivity for CIN2+ and CIN3+ lesions with care HPV™ while the specificity was better with the OncoE6 cervical test™[8]. However, till date, no systematic review has been performed for the POC or near-POC tests for HPV DNA or RNA detection in cervical samples. Such concise information is likely to prove useful to researchers for future directions in this field as well as to the policymakers in guiding the formulation and updation of the cervical cancer screening guidelines. This review aimed to summarize the currently available literature on POC tests for HPV-based cervical cancer screening and to briefly outline the emerging technologies in this field.

Methods

Search strategy: A systematic literature search was conducted from January 1, 2004 (after the approval of HPV tests for cervical cancer screening as a co-test) upto December 31, 2022, using the following search terms: 'cervical cancer', 'human papillomavirus (HPV)', 'cervical cancer screening', 'point of care testing', 'evaluation', 'near-patient',' and 'performance'. MEDLINE and Cochrane databases were searched for relevant articles. Reference lists of all the included articles were checked for additional relevant studies.

Inclusion criteria: Studies describing POC or near-POC HPV detection (DNA or RNA) for cervical cancer screening were included in this review. HPV detection tests that can be performed at the site of sample collection (generally a primary health centre in resource-constrained settings) and provide results on the same day are considered to be POC tests[9]. There were no restrictions in context to the type of study, population studied, sample type included or language of publication.

Exclusion criteria: Studies that described techniques not suitable for POC testing or requiring extensive laboratory set-up were excluded from the review.

The included papers were reviewed for the HPV detection method or technology described and grouped into the following categories: (*i*) commercially available tests, (*ii*) prospective candidates under trial (tests that have undergone limited testing for cervical cancer screening) and (*iii*) upcoming advances (technologies that are yet to be evaluated for cervical cancer screening).

Results

Commercially available point-of-care (POC) or near-point-of-care (POC) tests for human papillomavirus (HPV) detection: At the time of writing this review, there were four commercially available methods for POC HPV-based cervical cancer screening. However, none of these assays have yet received the US-FDA approval for use in cervical cancer screening.

careHPV™: The careHPV™ test was developed by Qiagen[8], as a low-cost alternative for resource-constrained countries. careHPV™ is a nucleic acid hybridization test with signal amplification that requires only low cost benchtop equipment and provides a qualitative result within ~2.5 h[10]. Numerous studies have demonstrated the comparability of careHPV™ results with HC2, both in clinical trials with well-trained staff in a laboratory set-up and for screening the general population as well as high-risk groups in resource-constrained settings[11-13].

careHPV™ has also been evaluated for use in cervical cancer screening using the self-collected vaginal samples. A recent meta-analysis reported the sensitivity of careHPV™ for the detection of CIN2+ lesions using clinician-collected samples as 88.1 per cent and specificity as 83.7 per cent[8]. For self-collected vaginal specimens, the sensitivity of careHPV™ was 73.6 per cent while the specificity was 88 per cent for CIN2+ lesions. However, the specificity of careHPV™ for CIN2+ lesions among women living with HIV (WLHIV) reduced to 58.8 per cent, attributed to the

higher number of transient HPV infections in this high-risk group. The specificity of HPV-based tests has also been linked to the prevalence of HPV infection in that a 10 per cent rise in prevalence leads to a drop in specificity by 8.4 per cent (8-8.8%)[14].

For cost-effectiveness of careHPV™ in cervical cancer screening, a micro-costing study by Shi *et al*[15] from China estimated an aggregated cost of US$ 7.49 for careHPV™ using self-sampling and US$ 7.95 for clinician-sampling. Another study by Levin *et al*[16] showed that the unit test cost of careHPV™ for screening at a national level was US$ 8.22 compared to US$ 45.89 for HC2 test. Cost-effectiveness ratio in this study estimated that the cost per year of life saved was US$ 50 for careHPV™-based cervical cancer screening performed even once in the lifetime of a woman.

OncoE6™ cervical test: The OncoE6™ cervical test from Arbor Vita Corporation provides qualitative results of elevation of E6 oncoprotein levels for HPV16 and HPV18 with partial HPV genotyping[17]. OncoE6 cervical test™ is a 'dipstick' test where cell lysates are conjugated with monoclonal antibodies to the E6 protein of HPV16/18 over a nitrocellulose paper strip. The conjugated complex is then detected using an enzyme substrate. The test has been developed to serve as an adjunct test with other clinical evaluations but is not meant to be used as a standalone screening test[18]. The systematic review by Kelly *et al*[8] reported the sensitivity of the OncoE6™ cervical test for the detection of CIN2+ lesions ranging from 31.3 to 42.4 per cent while the specificity was 99.1 to 99.4 per cent. The low sensitivity of this test has been attributed to the fact that about one-third of cervical precancerous lesions are caused by HR-HPV other than HPV16 or HPV18. Furthermore, the expression of E6 oncoprotein is linked to the potential of HPV-related lesions to progress over time. Hence, this test may miss the cervical lesions that have a lower progressive potential that do not overexpress E6 oncoprotein[19,20].

A study by Valdez *et al*[19] compared OncoE6™, careHPV™ and visual inspection with acetic acid (VIA) for the detection of cervical precancerous lesions in a population-based cervical cancer screening. This study reported a better sensitivity of careHPV™ (96.5% for clinician-collected and 81.6% for collected-vaginal samples) for detecting CIN3+ lesions compared to OncoE6™ (54.4%). However, the specificity of OncoE6™ (99.1%) was higher than careHPV™ (86.9% for clinician-collected and 86.6% collected samples)[19]. Similar results were reported in the study by Torres *et al*[21].

Xpert® HPV: GeneXpert® HPV is a cartridge-based test that employs nucleic acid amplification test (NAAT) for a qualitative result for 14 HR-HPV types with partial genotyping for HPV16 and HPV18/45 and can be performed on analyzers that are used for diagnosis of diseases such as tuberculosis and HIV by the same method. This assay can be performed as POC or near-POC using benchtop equipment and minimum hands-on sample preparation requirement and provides results within one hour[22]. This test has been validated so far only for samples collected in PreservCyt solution[23].

Field evaluation by Toliman *et al*[24] demonstrated an agreement between the HR-HPV, HPV16 and HPV18/45 detection with Xpert® HPV using self-collected samples as well as physician-collected samples. The availability of results in a short time, allowed triaging of HPV-positive women with VIA and same-day ablation therapy[24]. A recent large-scale study of Xpert® HPV test on self-collected vaginal samples demonstrated the sensitivity of this test to detect HSIL or worse lesions was 85.4 per cent while the specificity was 89.6 per cent with a negative predictive value of 98.9 per cent[25].

Inturrisi and Berkhof[26] assessed the tender-based pricing of HPV tests in Italy. Their study showed the unit price of Xpert® HPV as 33-42.3€ compared to 10.7€ for HC2 and 4.54€ for Cobas® 4800 HPV test[26]. The cost of the equipment may be a limiting factor in the widespread implementation of this assay for cervical cancer screening in low- and low-middle income countries. However, the cost of the assay would be significantly lower in settings where the equipment is already installed for the detection of tuberculosis or other diseases.

Truenat® HPV-HR: Truenat® HPV-HR assay has been developed by MolBio Diagnostics, India, entailing a real-time PCR-based detection of four HR-HPV types: 16, 18, 31 and 45 and providing results within one hour. A recent study[27] compared this assay with the US FDA-approved HC2 method. The sensitivity and specificity of Truenat® HPV-HR were reported as 97.7 per cent and 98.9 per cent, respectively, for the detection of HR-HPV (gold standard being HC2)[27]. However, further studies are mandated to validate these results as well as evaluate the performance characteristics of this assay for CIN2+ or CIN3+ detection. A limitation highlighted by this study was the number of samples

that can be analyzed at a time – the Truelab® Quattro allows for processing of four samples at a time which may prove to be a limiting factor in the field setting[27].

Prospective candidates under trial for point-of-care (POC) HPV testing: Apart from the aforementioned commercially available tests, there are a few upcoming technologies that are in various stages of development. These tests are tabulated in Table I[28-40].

Q- POC™: The Q-POC™ analyzer has been developed by Global Good partnering with QuantuMDx (Newcastle upon Tyne, UK) as a platform involving multiplex PCR and providing results within 30 min (Table I). As per the manufacturer, the probes used in the assay offer high specificity and the estimated cost for the equipment could be about US$ 650, with each cartridge costing about US$ 6.5-26[28]. The Q-POC™ HPV assay is currently being evaluated in a multi-site clinical study[41].

Lab-on-Chip assays: A lab-on-chip assay is essentially a miniature device based on microfluidics that can integrate one or more tests on a single chip. This technology offers several advantages such as lower cost, compact design, ease of use, faster results, lower sample volumes, reduction of human errors and higher sensitivity for the test under consideration. However, many such assays that seem to work well in the laboratory do not lend themselves to commercialization. The ethical and regulatory issues pertaining to this new technology are yet unsettled[42]. In the field of HPV-based cervical cancer screening, the following lab-on-chip assays are under development:

(i) The Biomedical Engineering Department at Boston University, USA, has developed an isothermal loop-mediated amplification (LAMP) technique with lateral flow strips as a POC testing for HPV16 (Table I). In a preliminary clinical validation, the group examined 10 cervical samples (five HPV positive and five HPV negative). There were two false positives attributed to the phenomenon of self-priming of the primers used in LAMP[29].

(ii) Wormald *et al*[30] have developed a lab-on-chip utilizing LAMP assay (using HPV DNA and (human telomerase reverse transcriptase [hTERT] RNA) coupled with ion-sensitive field-effect transistor sensors. The investigators tested this device on 10 cervical biopsies (5 benign, 5 malignant). HPV16 DNA was detected in 4/5 of the malignant and 2/5 of the benign tissues, while HPV18 DNA was seen in 1/5 each of the malignant as well as benign tissues. hTERT messenger RNA was detected in all the malignant and none of the benign samples. These results were concordance with the standard PCR. The authors propose that this assay would be clinically applicable to cervical brush samples as well[30].

(iii) Another lab-on-chip device developed by Zhu *et al*[31] (Table I) detects five HR-HPV types (16, 18, 31, 33, 45). The authors validated the device with 20 cervical samples, of which 16 tested positive for one of the five genotypes on the chip. This was confirmed by real-time PCR[31].

(iv) A LAMP-based nucleic acid amplification system for the detection of HPV16, 18, 39, 45 and 52 has been developed by Zhao *et al*[32]. The system was compared with quantitative PCR using HeLa cells–48 HPV-positive and 48 HPV-negative samples. The sensitivity and specificity of the LAMP assay were 100 per cent sensitive and 91.7 per cent, specific[32].

(v) Yin *et al*[33] built a smartphone-based smart cup platform for LAMP-based detection of HPV 16, 18 and 31 DNA. As a proof of concept, 15 cervical swab samples (3 HPV positive and 12 negative) were analyzed using this device, and results showed a 100 per cent agreement with real-time PCR. However, further validation in a larger study and inclusion of other high-risk HPV types is mandated for this device[33].

(vi) Smith *et al*[34] developed a paper-based signal amplification assay requiring a low-cost benchtop heater and involving seven steps to be performed by the user. The performance of this assay was assessed with 16 biobanked cervical swab samples (8 positive and 8 negative) showed 87.5 per cent sensitivity, 100 per cent specificity and 93.8 per cent accuracy (gold standard being careHPV™)[34].

HPV genotyping 9G Membrane test: A Korean group evaluated a 9G membrane test for potential use as a POC platform in cervical cancer screening. In their validation study[35], 550 cervical samples were analysed for five HR-HPV types – 16, 18, 31, 33 and 45. The results of 9G membrane test were in 100 per cent agreement with the sequencing results. Considering the PCR-based HPV detection as the gold standard, the sensitivity and specificity of the 9G membrane test were found to be 100 per cent[35]. This product was approved by the Korean FDA and commercialized by BMT Chip (*https://www.bmtchip.com/bbs/board.php?bo_table=products_en&wr_id=7*). The requirement of

Table I. Prospective candidate tests for point-of-care testing for human papillomavirus-based cervical cancer screening

POC HPV test method	Test principle	Test performance evaluation	Merits	Limitations
Q-POC[28]	Multiplex PCR Detects 13 HR-HPV types Up to 15 tests per day	Not yet reported	Results within 30 min Simple pre-processing	Costly equipment Analyzes one sample at a time Performance not reported yet
		Lab-on-a-chip		
Rodriguez *et al*[29]	Paper-based microfluidic system with LAMP that combines nucleic acid extraction, amplification and detection in a paper fluidic diagnostic chip to provide results in less than 1 h	2/5 false positives (5 HPV positive and 5 HPV negative tested)	Results within 1 h Low-cost Portable Limit of detection 10^4 total copies of viral DNA	External heat source required User intervention (pipetting steps) Developed for HPV16 only till now
Wormald *et al*[30]	Microfluidic cartridge-based LAMP with ISFET sensors using HPV DNA and *hTERT* mRNA A change in the *p*H of the solution due to DNA amplification leads to a voltage change that can be detected by ISFETs	Not reported for cervical smears (tested on cervical biopsies)	Results within 30 min Limit of detection 10^3 copies for *hTERT*	Not yet tested for cervical smears
Zhu *et al*[31]	Microfluidic chip integrating nucleic acid extraction, solid-phase PCR and genotyping	100% concordance with real-time PCR in 20 tested samples	Results within 1 h Simple, small and easy-to-use chip Includes 5 HPV genotypes - 16, 18, 31, 33, 58	Performance yet to be tested on larger number of samples
Zhao *et al*[32]	LAMP-based amplification using microfluidic system	Compared with HeLa cells - 100% sensitive, 91.7% specific	Results within 40 min Can perform up to 40 tests at once 5 HPV genotypes - 16, 18, 39, 45, 52	Not yet tested on clinical samples
Yin *et al*[33]	Smartphone-based smart cup with microfluidic chip LAMP-based HPV DNA detection Nucleic acid amplification followed by the capture of the colour signal by a smartphone camera and analysis using a customized app ('Hue Analyzer')	Good concordance with real-time PCR on 15 cervical samples	Results within 1 h 3 HPV genotypes - 16, 18, 31	Validation with larger number of samples required Inclusion of other HR-HPV types
Smith *et al*[34]	Paper-based hybrid capture detection of HPV DNA	Sensitivity 87.5% Specificity 100% Accuracy 93.8% tested with 16 biobanked samples	Results within 1 h Good sensitivity and specificity	Validation with large number of clinical samples not done yet Requires external heating device and multiple sample preparation steps
9G membrane HPV test[35]	Nucleic acid hybridization on 9G membrane (glass membrane lined with oligonucleotide probes and appended with 9 consecutive guanines) after external amplification	100% concordance with sequencing analysis on 550 clinical samples	Results within 30 min 5 HPV types - 16, 18, 31, 33, 45	Requires DNA extraction and amplification before application on the 9G membrane for hybridization

Contd...

POC HPV test method	Test principle	Test performance evaluation	Merits	Limitations
DNA-focussed digital microholography[36]	Silica-coated poly (methyl-methacrylate) microbeads used for DNA isolation followed by asymmetric PCR using a portable mini-PCR device Amplification product loaded onto the AIM-HPV device equipped with a 5-megapixel CMOS image sensor AIM-HPV device captures images of the PS bead, silica bead and PS-silica dimer counts that are analyzed by a deep learning algorithm to classify as positive or negative	Good correlation with Cobas HPV test	Can be used for POC testing with minimal training for the staff	DNA extraction and amplification to be performed before hybridization in the POC device Developed for HPV16 and HPV18 Need for on-board amplification, including other HPV types, and reagent lyophilization
		CRISPR		
Tsou *et al*[37]	CRISPR-Cas12a system in a lateral flow dipstick method for HPV detection in plasma	5/14 cancer-free subjects detected with HPV16 in plasma	Results within 5 min Offers a solution for plasma-based detection of HPV DNA	False positivity Requires improvement in efficiency of detection Evaluation in a larger sample size needed
Zhou *et al*[38]	CRISP/Cas12a coupled with RPA in microfluidic platform	Full-text not available	Results within 30 min Low-cost detection On-chip amplification Simple and fast detection in lateral flow dipstick format	Validation in a larger clinical sample set required
Zheng *et al*[39]	Cas13a/Cas12a dual-channel with multiplex RPA in a fluorescence-based assay	Sensitivity 95% Specificity 100% in a clinical evaluation of 55 samples	High sensitivity and specificity Accurate, convenient platform	Needs to incorporate nucleic acid-free extraction
C-ColAur[40]	Gold nanoparticle-based colorimetric assay *In-situ* formation of gold nanoparticles detected using a reducing agent (ascorbic acid)	Sensitivity 96.42% Specificity 71.42% in a study of 28 cancer and 14 normal samples	Rapid analysis No pre-processing required No high-end equipment required	Validation with a larger number of samples required

Note: Many of the mentioned tests are not commercially available yet/under the process of getting developed. POC, point-of-care; HPV, human papillomavirus; HR-HPV, high-risk-HPV; PCR, polymerase chain reaction; LAMP, loop-mediated isothermal amplification; ISFET, ion-sensitive field-effect transistor; AIM-HPV, artificial intelligence monitoring for HPV; PS, polystyrene; CRISPR, clustered regularly interspaced short palindromic repeat; RPA, recombinase polymerase amplification; CMOS, complementary metal oxide semiconductor

DNA extraction and amplification, however, might limit the current use of this assay for cervical cancer screening in field settings.

DNA-focussed digital microholography: An interesting technology – 'DNA-focussed digital microholography' – has been developed by Pathania *et al*[36]. The device was validated on 28 cervical samples and seven cervical biopsies, comparing the results with the reference test (Cobas® 4800 HPV test) for HPV16 and HPV18, showing a good correlation in HPV signals between pairs of cervical brush and biopsies (Pearson correlation coefficient r=0.93). A full concordance was found between the AIM-HPV assay and the Cobas® 4800 HPV test. The authors intend to improvise the device by integrating an isothermal amplification step, including other HR-HPV types and reagent lyophilization for longer storage to allow for translation into clinical practice[36].

Clustered regularly interspaced short palindromic repeats (CRISPRs): (CRISPR)-based detection has shown great promise in the molecular diagnosis of various diseases. However, the technique requires expensive equipment and trained workforce. Tsou *et al*[37] have developed a lateral flow dipstick method with HPV16/18-targeting CRISPR RNA and custom ssDNA-FAM quencher reporter. This technique was evaluated in plasma samples of 15 individuals with cervical cancer and 14 cancer-free individuals. The CRISPR-Cas12a system could detect HPV16 and HPV18 in 13/14 and 3/10 individuals with cervical cancer, respectively. However, HPV16 was also detected in 5 out of the 14 individuals who were cancer-free. This false positivity has been explained by the presence of inflammation or infection that could facilitate the viral spread into the blood circulation, and the possibility of an off-target effect of CRISPR RNA, that is the presence of sites on the RNA with partial homology[37].

CRISPR/Cas12a was coupled with recombinase polymerase amplification in a fluorescence-based HPV detection system for HPV16 and HPV18 by Zhou *et al*[38]. This method, however, requires validation in a larger number of clinical samples.

The CRISPR system has been used with multiplex recombinase-aided amplification in a portable fluorescence-based assay designed by Zheng *et al*[39]. A clinical evaluation in 55 samples (7 HPV16/18 co-positive, 10 HPV16 positive, 10 HPV18 positive and 28 HPV negative) gave a sensitivity of 95 per cent and a specificity of 100 per cent[39]. CRISPR-based assays are not available commercially as of now.

Nanoparticle-based assays: Appidi *et al*[40] developed a gold nanoparticle-based colorimetric assay for cervical cancer screening (C-ColAur). In this validation study, 27 of the 28 cancerous samples and 10 of the 14 normal cervical samples were identified correctly, giving a sensitivity of 96.42 per cent and specificity of 71.42 per cent, suggesting its potential utility as a screening test (Table I). Studies with a larger number of samples, including cervical precancerous lesions are, however, imperative to validate the utility of this system in cervical cancer screening.

Upcoming advances in the field of point-of-care (POC) human papillomavirus (HPV) detection: In addition to the above-mentioned advances in the field of cervical cancer screening, a few more promising methods/technologies are under development that could be accelerated to clinical evaluation in the coming years.

Paper-based devices: A 'multiplex autonomous disposable NAAT (MAD-NAAT)' has been developed as a POC paper-based instrument-free network device that can provide qualitative results (Table II[43-49]). The prototype has been evaluated for the detection of methicillin-resistant *Staphylococcus aureus* in nasal swab specimens[43]. This assay is intended to be evaluated for cervical HPV DNA detection, for which optimization of cervical sample preparation might be needed.

A portable battery-powered platform for one-pot amplification and detection of fluorescence has been designed by Mei *et al*[44]. This device was tested for the detection of SARS CoV-2 RNA with a limit of detection as low as 4×10^2 copies per ml[44]. This technology holds promise for HPV DNA detection. However, the manual steps, requirement of cold storage for enzymes and size of the reader are likely to make this less suitable for field settings with untrained healthcare workers.

Serum or plasma-based tests: Abviris Deutschland GmbH, Ahrensburg, Germany, has marketed a product, Prevo-Check®, a competitive immunoassay for a qualitative detection of antibodies against HPV16 L1 in serum. So far, this product has received CE-IVD for the detection of anal and oropharyngeal cancers only. A study by Blatt *et al*[45] reported this assay to have a sensitivity, specificity and accuracy of 100 per cent for oral SCC. However, it is yet to be evaluated

Table II. Upcoming advances in point-of-care testing for human papillomavirus-based cervical cancer screening

Technique	Test principle	Test performance evaluation	Merits	Remarks
		Paper-based devices		
MAD-NAAT[43]	Isothermal amplification followed by detection using streptavidin-labelled gold nanoparticles	Not yet reported	Results within 1 h Dry storage of the reagents along with a buffer for rehydration Use of isothermal amplification with the release of amplicons to a LFS for detection	Yet to be evaluated for cervical HPV DNA detection
Paper-based nucleic acid enrichment[44]	Sample collection and lysis, paper-based enrichment of nucleic acids, RPA reaction, followed by result reading in a rechargeable portable reader		Results within 30 min	Manual steps for nucleic acid enrichment, reagent addition for amplification and result reading
		Serum or plasma-based tests		
Prevo-Check[45]	Competitive immunoassay for a rapid qualitative detection of antibodies against HPV16 L1 in the serum		Good sensitivity and specificity in oral SCC	Yet to be evaluated in cervical cancer screening
Obahiagbon *et al*[46]	Excitation module with LEDs that excite the sample in nitrocellulose membranes mounted on glass slides Signal readout module contains an emission filter and photodiodes to capture the fluorescence signal			Not yet tested for cervical smears
Keyvani *et al*[47]	Two modules: A passive plasma separator to isolate the plasma and an electrochemical biosensor for HPV16 cDNA detection		Relatively non-invasive nature Use of a passive plasma separation method, obviating the need for a centrifuge	Yet to be evaluated in cervical cancer screening
MCE[48]			Detection within 3 min after amplification LoD 10^2 cells/ml	
Graphene oxide-based biosensor[49]			Linear nature of detection in the concentration range of 1 pM-1 µM of DNA	

Note: Many of the mentioned tests are not commercially available yet/under the process of getting developed. MAD-NAAT, multiplex autonomous disposable nucleic acid amplification test; LEDs, light-emitting diodes; SCC, squamous cell carcinoma; LoD, limit of detection; LFS, lateral flow strip; RPA, recombinase polymerase amplification; MCE, microchip electrophoresis

in screening for cervical cancer and precancerous lesions.

A compact multiplex fluorescence-based platform has been designed by Obahiagbon *et al*[46]. This platform was tested on two plasma samples – one HPV16E7 antibody positive and another negative as a proof-of-concept. Since the system is compact, field trials for the detection of HPV16 and HPV18 antibodies for cervical cancer screening may be undertaken soon.

Keyvani *et al*[47] have used a graphene oxide-based electrochemical sensor in an integrated microfluidic assay for the detection of circulating HPV DNA (cDNA) in the plasma. However, the limit of detection of DNA by this device was higher than the average range found in individuals with cervical cancer. Hence, either an amplification step needs to be interposed or the sensitivity of the biosensor enhanced for better detection.

Other techniques: Fan *et al*[48] used a simple, rapid and automated microchip electrophoresis (MCE) for the detection of HPV16 and HPV18. The MCE facilitates the detection of the DNA product within three minutes post amplification. The authors used CaSki cells for HPV16 DNA and HeLa cells for HPV18 DNA. The sensitivity of MCE for the detection of HPV16 and HPV18 was found to be 10^2 cells/ml[48]. The MCE detection system has not yet, however, been validated in clinical samples. There is also a need for integration of the DNA amplification with MCE to devise a complete handheld HPV detection system.

An electroanalytical biosensor utilizing the graphene oxide-DNA hybrid method was devised by Rawat *et al*[49]. This technology needs to be further refined and developed as a portable device for POC HPV DNA-based cervical cancer screening.

Other cost-effective strategies for resource-limited settings:

Urine-based human papillomavirus (HPV) detection: Urine-based detection of HR-HPV by Das *et al*[50] in 1992 has recently garnered attention as a non-invasive and simple alternative for population-based cervical cancer screening. Urine HPV detection is based on the premise that the first-voided urine in a female contains exfoliated cells from cervical epithelial lesions which help in detecting HPV infection[50,51]. Although studies including a recent meta-analysis demonstrate a good sensitivity and specificity for HR-HPV detection in urine samples compared to cervical samples, none of the commercially available HPV assays have so for been clinically validated for urine samples[52]. Adaptation of urine self-collection for POC HPV detection is a difficult task due to technical issues. A recent study[53] attempted to use Xpert® HPV in urine samples from 40 women (30 from colposcopy clinic and 10 undergoing routine screening) and demonstrated a sensitivity of 66.7 per cent and a specificity of 86.7 per cent when compared with cervical samples[53].

Paper smear method of cervical sample collection: In view of the non-accessibility of appropriate sample transport facilities in hard-to-reach areas, a 'paper smear' method of cervical sample collection was developed by Kailash *et al*[54] where the cervical scrape is collected on a sterile Whatman 3MM filter paper cut in the size of a standard glass slide, air-dried and transported to the laboratory for PCR-based HPV detection in a single tube without extracting DNA separately. The quality and quantity of DNA extracted from this sample were shown to be comparable to those obtained from the same women and stored in phosphate-buffered saline at −70°C. This technique offers an alternative for the implementation of HPV-based cervical cancer screening in remote areas by effectively transporting samples to a reference laboratory. However, the advantages of POC or near-POC HPV testing, such as rapid results and screen-and-treat approach, are not feasible with this method.

Discussion

Cervical cancer remains a major public health concern globally, amounting to 6,04,127 new cases and the 3,41,831 deaths in year 2020[55]. In May 2018, the World Health Organization issued a 'Call for Action' for cervical cancer elimination by 2030. As a part of the strategy for achieving this goal, at least 70 per cent of the eligible women should be screened using a high-performance test twice in their life (at 35 yr and 45 yr)[56]. Among the available modalities for cervical cancer screening, HPV DNA-based tests have shown higher sensitivity for detecting CIN2+ lesions. However, the majority of the commercially available US-FDA-approved HPV detection tests are costly with the requirement of an elaborate laboratory set up and trained workforce. These limitations have hampered the widespread utility of HPV-based cervical cancer screening in resource-constrained countries. Hence, various researchers have been attempting to develop a cost-effective, easy-to-use, rapid and efficient POC test for HPV-based cervical cancer screening.

A modelling study by Campos *et al*[22] indicated that POCT-based cervical cancer screening with adequate linkages to treatment might be worthy of investment, especially in countries where the loss to follow up is relatively high[22]. Camara *et al*[57] reported a high level of acceptability of self-collection of cervical samples for POC HPV testing by GeneXpert™ and the screen-and-treat approach in the Pacific Islands paving the way for further research on POC HPV-based tests for resource-constrained countries.

Although the field of POC tests for HPV detection has generated a significant interest, a few criteria need to be kept in mind while developing these technologies such as: *(i)* adequate validation process of newer tests including all the molecular steps, especially in a screening population, *(ii)* validation of HPV test in women who have undergone treatment for cervical precancerous lesions or cancer, *(iii)* validation protocol for self-collected samples and *(iv)* validation

of HPV genotyping tests along with the decision on the comparator test[58]. Although careHPV™ and Xpert® HPV tests are commercially available for cervical cancer screening, their current cost imposes a limitation on their scalability for population-based screening, especially in low-resource countries[8]. Ideally, a test with a clinical sensitivity of 90-95 per cent and a specificity of 90-98 per cent and cost of not more than US$ 5 should be targeted for cervical cancer screening[59]. The traditional nucleic acid extraction and amplification techniques mandate a laboratory set up and complex instrumentation entailing high cost. The need of the hour is a test with a limited requirement of equipment and minimal sample preparation or hands-on involvement so that semi-trained healthcare providers may be able to perform the task. Technologies such as (LAMP) perform nucleic acid amplification at a single temperature (isothermal) and thus require only a simple single-temperature heater. The combination of LAMP technique with paper-based microfluidic system in assays, such as those devised as Rodriguez, Wormald, and other authors, hold promise in yielding an actual POC HPV test for resource-constrained countries. Researchers hence need to incorporate multiple HPV genotypes in the same device along with a possibility of genotyping, for HPV16, HPV18/45 and similar HPV oncogenic types.

The combination of isothermal nucleic acid amplification with the CRISPR/Cas technique may be explored to provide a simple, low-cost and portable POC testing device for HPV-based cervical cancer screening in the future.

The recent advances in the field of nanotechnology have allowed for the development of nanobiosensors for various purposes. Nanobiosensors, usually made from inert materials such as gold, work on the principle that binding of the analyte of interest to the biosensor leads to a change in the physicochemical signal that can be converted by a transducer into an electric signal[60]. Nanobiosensors are being developed for POC detection of various cancers such as breast and ovarian[61]. In the field of cervical cancer screening, gold nanoparticle-based and graphene oxide-based nanoparticles have been explored for development of rapid assays[38]. However, these techniques require further refinement to achieve a rapid, simple, efficient and inexpensive POC test for HPV-based screening.

The science of microfluidics has been evolving rapidly with the integration of artificial intelligence (AI), making it intelligent microfluidics. AI and machine learning can be utilized to optimize the circuit modelling of the microfluidics, fabrication and 3D printing and provide control through automation over the fluid flow, temperature, particle manipulation and so on. Nanoparticle-based sensors can also be optimized using AI. Similarly, AI and machine learning can be integrated with signal recognition and analysis for result generation in new-age diagnostic systems[62]. The field of HPV detection has already witnessed the advent of AI-based system developed by Pathania *et al*[36], and this is only likely to grow and expand further.

This review highlights that, there are a few commercially available HPV detection methods suitable for POC testing and many others at various stages of development and testing to develop and validate a suitable POC test for HPV-based cervical cancer screening particularly for resource-limited countries. In our opinion, there is an imperative need for inter-group, inter-institutional and inter-country collaborations on this topic to make optimum use of the best available technology and scientific minds in devising such a test that fulfils the requirement of a cervical cancer screening modality with robust performance characteristics.

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