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REVIEW

Advances in technologies for cervical cancer detection in low-resource settings

Kathryn A. Kundrod[a], Chelsey A. Smith[a], Brady Hunt[a], Richard A. Schwarz[a], Kathleen Schmeler[b] and Rebecca Richards-Kortum[a]

[a]Department of Bioengineering, Rice University, Houston, TX, USA; [b]Department of Gynecologic Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

ABSTRACT

Introduction: Cervical cancer mortality rates remain high in low- and middle-income countries (LMICs) and other medically underserved areas due to challenges with implementation and sustainability of routine screening, accurate diagnosis, and early treatment of preinvasive lesions.

Areas covered: In this review, we first discuss the standard of care for cervical cancer screening and diagnosis in high- and low-resource settings, biomarkers that correlate to cervical precancer and cancer, and needs for new tests. We review technologies for screening and diagnosis with a focus on tests that are already in use in LMICs or have the potential to be adapted for use in LMICs. Finally, we provide perspectives on the next five years of technology development for improved cervical cancer screening and diagnosis in LMICs.

Expert opinion: Innovation toward improved molecular and imaging tests is needed to enable effective, affordable see-and-treat approaches to detect and treat cervical precancer in a single visit. Current molecular tests remain too complex and/or costly for widespread use. Especially with imaging tests, decision support may improve performance of new technologies.

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1. Introduction

1.1. Cervical cancer screening and diagnosis

Cervical cancer is preventable with high-risk human papillomavirus (HPV) vaccination as well as screening, diagnosis, and treatment of preinvasive lesions. However, approximately 570,000 people are diagnosed and 311,000 people die of cervical cancer annually [1]. Routine screening programs implemented in high-income settings have been effective in reducing cervical cancer mortality. In low- and middle-income countries (LMICs), where screening, diagnosis, and treatment are often challenging to implement, cervical cancer mortality rates remain high; nearly 90 percent of cervical cancer deaths occur in LMICs [2]. The incidence and mortality rates of cervical cancer depend largely on socioeconomic status, availability of preventive health services, resource levels [3], and HIV prevalence [4]. Without significant intervention, especially in LMICs, cervical cancer deaths are expected to rise by almost 25% globally between 2014 and 2024 [5]. The global burden of cervical cancer is expected to increase in LMICs to 95% of overall cervical cancer deaths by 2030 [6]. Methods of intervention include primary prevention by prophylactic high-risk HPV vaccination, the etiologic agent for nearly all cervical cancers, and secondary prevention through screening, diagnosis, and treatment of cervical abnormalities.

While there is a safe and effective prophylactic vaccine to prevent infection with high-risk HPV, there are currently low rates of vaccine uptake globally [7]. Improving HPV vaccination rates through carefully designed and funded programs should be prioritized as a strategy to reduce cervical cancer incidence in LMICs. Vaccination is particularly useful in reaching more remote populations and in protecting younger generations. However, several generations of women have already been exposed to high-risk HPV and therefore will not benefit from HPV vaccination, which does not treat pre-existing infections and related abnormalities. Until we achieve universal vaccination, there is a critical need for improved screening, diagnostic, and therapeutic tools for at least several generations.

In this review, we first describe the standard of care for screening and diagnosis in high- and low-resource settings; we review biomarkers associated with cervical precancer and cancer; and we discuss needs for new tests. We then discuss promising new technologies that could increase access to cervical cancer screening and diagnosis in LMICs. Finally, we discuss the need for continued innovation to reduce rates of cervical cancer incidence and mortality globally.

1.2. Current methods for cervical cancer screening and diagnosis

Several technologies and methods are used for cervical cancer screening and diagnosis. In this section, we first describe the gold standard diagnostic test against which the clinical performance of new technologies is measured. We then describe the currently recommended practices for cervical screening and diagnosis in high- and low-resource settings.

CONTACT Rebecca Richards-Kortum rkortum@rice.edu Rice University, Bioengineering, MS-142, Houston, TX 77005, USA
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Article Highlights

- Cervical cancer is preventable with human papillomavirus (HPV) vaccination, as well as screening, diagnosis, and treatment of precancerous lesions. However, cervical cancer incidence and mortality rates remain high in low-resource settings, where there is a critical need for accessible screening and diagnostic tools.
- Guidelines for screening, diagnosis, and treatment differ for high- and low-resource settings; we review example guidelines for both settings.
- A number of biomarkers are strongly correlated to cervical precancer and cancer progression, including HPV DNA, mRNA, and oncoproteins.
- We review molecular testing approaches for cervical cancer screening. To be useful globally, new molecular screening tests for cervical cancer must offer high clinical sensitivity at low cost. We compare performance of commercially available technologies that are either currently in use in low-resource settings or utilize a central technology that could be adapted for use in a low-resource setting; we also review promising technologies in development.
- We review optical testing approaches for cervical cancer diagnosis. New optical diagnostic tests should incorporate real-time feedback and automated image analysis. We highlight devices that perform mobile colposcopy and *in vivo* microscopy, as well as machine learning algorithms that could be used for decision support.
- High-performance, low-cost technologies offer the promise of a new standard of care for cervical cancer screening in low-resource settings, including accurate screening, diagnosis, and treatment within a single visit. New technologies with particular promise include mRNA testing, self-sampling, and machine learning-based decision support.

1.2.1. Gold standard for cervical cancer diagnosis

The gold standard for the diagnosis of both cervical dysplasia and invasive cancer is histopathologic examination of biopsied specimens to identify premalignant and malignant conditions of the cervix. In this process, a pathologist examines the biopsied epithelium of the cervix and classifies it according to the fraction of the epithelial layer that displays abnormal cellular morphology. For squamous epithelium, cervical intraepithelial neoplasia (CIN) 1 or low-grade squamous intraepithelial neoplasia (LSIL) is when a third or less of the epithelium has undergone cellular changes; CIN2 and 3 or high-grade squamous intraepithelial neoplasia (HSIL) is when greater than one-third of the squamous epithelium displays abnormal cellular morphology. Adenocarcinoma-in-situ (AIS) is when the glandular cells show abnormal morphology. Cancer is diagnosed when invasion is noted in the squamous epithelium (squamous cell carcinoma) or glandular epithelium (adenocarcinoma). If left untreated, CIN2 or more severe diagnoses (referred to as CIN2+ diagnoses) can progress to invasive cancer and therefore are commonly treated by ablation or excision to prevent progression [5]. More detailed definitions of tumors and their precursors are outlined in the World Health Organization (WHO) Blue Book [8]. Screening and diagnostic tests are generally evaluated in terms of clinical sensitivity and specificity relative to the gold-standard of biopsy-proven CIN2+; the sensitivities and specificities reported throughout this article follow this convention.

1.2.2. Standard of care screening and diagnosis in high-resource settings

In high-resource settings, the standard of care for cervical cancer screening includes cervical cytology and/or high-risk HPV DNA or RNA testing, as the vast majority of cervical cancer is caused by infection with HPV. Cytology, commonly referred to as the Pap test, involves examining the morphology of exfoliated cervical cells under a microscope and generally has a low sensitivity (53–55.4%) and high specificity (84.2–94.5) [9–12]. Cytology performance varies greatly, even within the United States due to interpretative variability [13]. In low-resource settings, the challenge of achieving high-quality cytology is greater because of a lack of medical capacity and even logistical capacity to get high-quality reagents into the country. Therefore, sensitivity may be even lower in low-resource regions than in higher-resource settings, where it is at best moderate, because validated Pap staining and/or liquid-based cytology is not available. As such, quality assurance of cytology is important to achieve similar preventive impact on cervical cancers compared with validated cytological methods. To compensate for low sensitivity in the United States, cytology testing efficacy comes from repeated, regular screening [14]. HPV DNA testing, in comparison, has relatively higher sensitivity (90.2–96.1%) and lower specificity (84.2–94.5%) in screening populations [9–11,15].

The latest recommendations by the United States Preventive Services Task Force (USPSTF) include cytology testing every three years for women aged 21–29 years. For women aged 30–65 years, testing by cytology every three years, HPV testing every five years, or co-testing with cytology and HPV every five years for women is recommended [16]. Guidelines for management of abnormal screening results have also been published by the ASCCP; these guidelines clarify when patients should be sent for confirmatory testing versus one-year follow-up based on initial screening results [17]. An example of a management algorithm for use with primary HPV screening in high-resource settings is shown in Figure 1.

Screening generally occurs less frequently in other high-resource settings compared with the United States while retaining good outcomes. The American Society of Clinical Oncology (ASCO) published global screening guidelines for four tiers of resource levels – maximal, enhanced, limited, and basic. Screening intervals for each resource level are as follows: every five years between the ages of 25–65 (maximal); every 5 years between the ages of 30–65 or every 10 years following consecutive negative tests at 5-year intervals (enhanced); every 10 years for ages 30–49 (limited); and one to two times per lifetime between the ages of 30–49 (basic) [18].

Country-level guidelines for high-resource settings generally follow the ASCO maximal recommendations. For example, cervical cancer screening by HPV testing occurs once every five years for women between 30 and 60 years old in the Netherlands and in Finland [19,20]. Similarly, in Australia, screening occurs once every five years between the ages of 25 and 74 by HPV testing [21]. Norwegian health authorities recommend screening by Pap testing once every three years between the ages of 25 and 69, though they will be switching to primary HPV testing in the near future [22]. In Sweden, primary screening is recommended by cytology for women ages 23–29 and by HPV for women between the ages of 30 and 64. The recommended interval between negative screening tests is 3 years for women ages 23–50 and 7 years for women ages 51–64 [23].

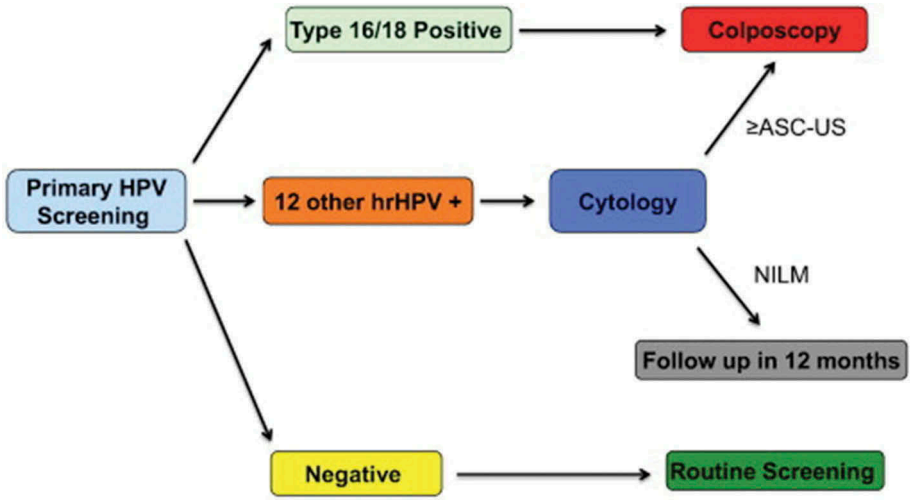

Figure 1. Example of a cervical cancer screening and management algorithm when using primary HPV screening. hrHPV: high-risk HPV; ASC-US: atypical squamous cells of undetermined significance; NILM: negative for intraepithelial lesion or malignancy. Reproduced from [17] with permission from Elsevier.

United States cervical cancer screening expenditures are high, due in part to frequent, unnecessary screenings of healthy women, though portions of the population remain unscreened. For example, rates of cervical cancer in the Rio Grande Valley of Texas are 55% higher than the rest of the United States, and only 12.9% of eligible, uninsured women are screened [24]. More broadly, approximately 20% of eligible women in the United States are not up-to-date on screenings [25], and women who have never been screened account for 50% of cancer cases [26].

In the United States, the determination of which tests are cleared for use with published guidelines is made by the Food and Drug Administration (FDA). To date, the FDA has approved HPV DNA and RNA tests for cervical cancer screening applications. Two HPV DNA tests are cleared for use in primary screening; the remaining DNA tests and one RNA test are cleared for use in primary screening if performed in conjunction with cytology (referred to as co-testing) in women over 30. Additionally, multiple HPV partial genotyping assays have been approved by the FDA for primary screening; to date, no extended genotyping tests are FDA approved [27,28]. Commercial molecular tests, including those that are FDA-approved, and emerging technologies are described in Section 2.

Screening by cytology requires infrastructure to obtain, store, and transport a cytology specimen, as well as a skilled technician or automated reader to process the sample. Currently FDA-approved HPV tests similarly require significant laboratory and transportation infrastructure and/or skilled technicians. Innovations in digital cytology [29] and HPV testing [30,31] could increase access to standard-of-care practices in low-resource settings, although the technical complexity, infrastructure requirements, and cost are significant barriers.

A positive screening test result triggers standard diagnostic procedure, including colposcopy and biopsy. In colposcopy, a trained provider examines the cervix, using a colposcope, which is a low magnification optical microscope. Visually abnormal areas are biopsied, excising small samples of cervical tissue for histopathological examination. Given the reliance on highly trained providers, it is challenging to scale diagnostic procedures in low-resource settings. Newer optical technologies may allow for automated visual evaluation, reducing the need for extensively trained personnel, and therefore more easily scaling in low-resource settings [32].

1.2.3. Standard of care screening and diagnosis in low-resource settings

Many barriers to implementing cervical cancer screening programs exist in low-resource settings, including but not limited to: lack of trained providers, lack of laboratory supplies, lack of laboratory infrastructure, socio-religious and cultural barriers to pelvic examination, unsustainable rates of overtreatment, and limited physical access to patient populations [33]. Decisions regarding appropriate screening and diagnostic technologies are made primarily based on available resources. For example, the 2013 World Health Organization guidelines for implementing cervical cancer screening in a low-resource setting are shown in Figure 2.

In addition to the screening test options available in high-resource settings, visual inspection with acetic acid (VIA) and by Lugol's Iodine (VILI) have been recommended for use in LMICs due to their low cost and limited infrastructure requirements. VIA and VILI involve applying acetic acid or Lugol's Iodine, respectively, to the cervix and observing color changes, which indicate precancerous or cancerous lesions. In a large study of a screening population in rural India, sensitivity and specificity of VIA were reported as 41.4% and 94.5%, respectively. These methods are highly dependent upon user training and environmental considerations, such as lighting conditions. Therefore, VIA and VILI have highly variable clinical performance. In one report, for example, the range of VIA sensitivity was 55–96% and specificity was 49–98%, and the range of VILI sensitivity was 44–98% and specificity was 75–91% [34]. Additional study with pathologic endpoints is needed to determine the true sensitivity and specificity of VIA and VILI but have been challenging to perform in low-resource

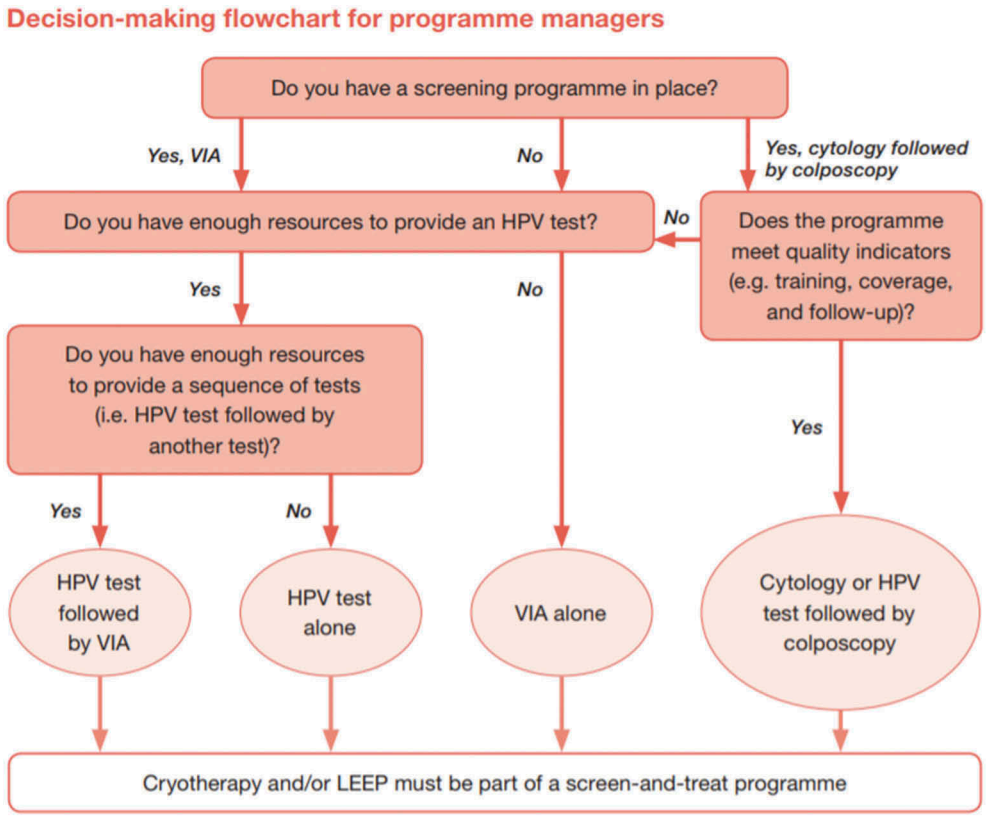

Figure 2. Decision-making flowchart for implementing screen-and-treat programs in low-resource settings. Decisions to implement HPV testing, VIA, cytology, and colposcopy for screening are made primarily on the basis of available resources. HPV: human papillomavirus; VIA: visual inspection by acetic acid. Reproduced with permission from [5].

settings where these tests are in use because of the lack of medical capacity and infrastructure to do colposcopy and pathology. While visual inspection tests are inexpensive and have limited supply chain requirements, Silkensen *et al* argue significant scale-up challenges, problematic accuracy, and insufficient reproducibility will limit their use moving forward [35].

Where feasible, objective tests with improved performance, like HPV testing, are recommended for use in LMICs; however, technology to support HPV DNA testing remains inaccessible in much of the world. Self-sampling for HPV DNA tests could help reduce barriers to screening program implementation in LMICs. Socio-religious and cultural barriers or unpleasant subjective experiences including discomfort with conventional physician-collected swabs can reduce compliance with cervical cancer screening programs. However, recent studies have shown good agreement between self-collected vaginal swabs and physician-collected cervical swabs for HPV DNA testing [36]. In addition, self-collection is strongly accepted and preferred according to a meta-analysis with nearly 20,000 women from 24 countries [37]. Technologies for self-collection of cervical samples have previously been reviewed [38]. Self-sampling could help remove barriers to HPV screening in LMICs without compromising test performance and could reduce the total time required during a screen-and-treat visit [39].

Similarly to the challenges faced in implementing screening programs in LMICs, availability of highly trained personnel and infrastructure often limit the accessibility and performance of diagnostic follow-up to positive screening tests. When available, colposcopy and biopsy are used for diagnosis in LMICs. When diagnosis is not available, screen-and-treat programs are implemented. Screen-and-treat programs include a screening test, such as VIA, and immediate treatment by cryotherapy or loop electrosurgical excision procedure (LEEP) of any positive-appearing cervical tissue. With currently available technologies, these programs may lead to overtreatment due to poor specificity [40].

1.3. Relevant biomarkers for cervical cancer screening

Molecular cervical cancer screening tests can target a number of clinically relevant biomarkers, primarily relating to HPV infections. Virtually all cases of cervical cancers are caused by HPV, a virus that integrates within the genome of host cells to disrupt normal cellular function. While there are over 200 types of HPV, only 14 are considered carcinogenic, or high-risk [41]. Several biomarkers related to high-risk HPV correlate to infection and, in some cases, progression toward cancer.

Here, we discuss biomarkers that are detected in FDA-approved tests, as well as biomarkers that are being explored in newer technologies for cervical cancer screening, making

them good tests to rule out the possibility of disease. Specifically, we discuss HPV DNA, RNA, oncoproteins, and genotyping, as well as other cervical cancer biomarkers.

1.3.1. HPV DNA

Tests that detect high-risk HPV DNA have high negative predictive values (NPVs) of over 98% for cervical precancer [42–44]. HPV DNA tests have high sensitivities (90.2–96.1%) and lower specificities (84.2–94.5%) in screening populations [9–11,15]. With low rates of false negatives, HPV DNA testing is often used as a first line screening test for cervical precancer and cancer. However, the Centers for Disease Control and Prevention (CDC) estimates that 90% of HPV infections are cleared within two years [41]. Therefore, confirmatory diagnosis for cervical precancer or cancer is necessary after an HPV DNA screen to avoid overtreatment. The risk of overtreatment associated with HPV DNA testing is lower in older patients, as rates of transient infections tend to decrease, and rates of type-specific persistence, which is required for cancer progression, tend to increase with age [45].

1.3.2. HPV mRNA

While the presence of HPV DNA indicates an infection, progression to cancer occurs when the infection persists, the viral genome integrates, mRNA overexpression of oncogenes begins, and oncoproteins are produced. mRNA overexpression of the E6 and E7 genes is the precursor for the production of E6 and E7 oncoproteins, which interfere with tumor suppressors p53 and pRB, respectively [46]. Therefore, evaluation of HPV E6 and E7 mRNA overexpression provides a more accurate assessment of risk of progression to cancer compared with HPV DNA. HPV mRNA testing has been shown to have comparable sensitivity and improved specificity for biopsy-proven CIN2+ compared with DNA testing [47]. Despite its advantages, current high-risk HPV mRNA tests remains too costly and complex for implementation in low-resource settings.

1.3.3. HPV oncoprotein

Like HPV mRNA, E6 and E7 oncoprotein detection has been shown to have high specificity for pre-cancer and cancer [48,49]. Both E6 and E7 are involved in the progression of HPV infection into precancer and cancer. Up-regulation of these proteins is needed for malignant conversion of HPV-infected cells, and over-expression implies high risk of progressive disease [46]. While oncoprotein detection improves specificity, sensitivity is generally lower than DNA or mRNA detection [48].

1.3.4. HPV genotyping

A newer area of focus is on partial or extended HPV genotyping. Each high-risk HPV type is associated with a different rate of cancer progression, so incorporating extended genotyping into primary screening may help in assessing the risk of progression for individual patients [50]. Genotyping may also help to monitor the persistence of type-specific infections, which are required for cancer progression, in individual patients.

On a population level, genotyping may be useful to incorporate into first-line screening to monitor changes in genotype prevalence, especially in response to increased vaccination rates. Currently, HPV types 16 and 18 cause over 70% of cervical cancers; these two types are covered by all commercially available, FDA-approved HPV vaccines [51–54]. Initial data about the impact of quadrivalent vaccination, which covers low-risk types 6 and 11 in addition to 16 and 18, have shown an 89% decrease in the detection of the HPV types included in the vaccine among vaccinated populations compared with a 34% decrease in unvaccinated populations, indicating a herd immunity effect [55]. As vaccination rates increase, it remains unclear if there will be a shift in the most prevalent oncogenic HPV types and how this will affect screening recommendations.

1.3.5. Other biomarkers

Additional biomarkers associated with cellular changes are also being investigated for use in screening tests. $p16^{ink4a}$ is a biomarker associated with progression to cervical precancer. Specifically, $p16^{ink4a}$ is a cyclin-dependent kinase inhibitor associated with E7 oncoprotein production [56]. p16 is reportedly expressed at high levels in CIN2+ and infrequently detected in benign tissue [57]. Recent studies pairing detection of $p16^{ink4}$ with Ki-67 show improved performance over traditional cytology or $p16^{ink4}$ alone. Currently, the dual $p16^{ink4}$/Ki-67 stain is used as triage after primary DNA testing [56,58,59]. Host methylation and viral DNA methylation also reflect cancer progression and are being investigated for triage after primary HPV testing [60–64]. Methylation analysis of two particular host genes, MAL and miR-124–2, have been shown to be non-inferior to cytology triage in a study of over 12,000 women [62]. In a small clinical study (n = 201), GynTect host methylation assay, which targets six DNA regions, produced positive results for all women who had cervical cancer, 61.2% of CIN3, 44.4% of CIN2, and 20.0% of CIN1 cases [63]. Further, DNA methylation of viral late regions (e.g. L1) has been shown to correlate to disease progression and has been evaluated in screening and triage settings [65–67]. One triage classifier, S5, detects DNA methylation of viral late regions from HPV genotypes 16, 18, 31, and 33 as well as the promoter region for human gene *EPB41L3* [65]. Relative sensitivity and specificity were assessed in a study of 15,744 women compared with the established triage method, which included liquid-based cytology (LBC) and HPV testing. For CIN2/3, relative sensitivity and specificity of the S5 classifier were 76% and 44%, respectively, and of LBC were 51% and 67%, respectively. For CIN3, relative sensitivity and specificity of the S5 classifier were 93% and 42%, respectively, and of LBC were 61% and 64%, respectively. While the S5 classifier did not show improved specificity over LBC, it did show high baseline sensitivity for CIN3, leading the authors to conclude it could be useful in simplifying existing triage algorithms [66]. Other promising biomarkers undergoing validation or clinical evaluation include proteins involved in cell cycle aberrations and miRNAs [56]. While these biomarkers do not yet have clinically validated tests, they have potential for use in the development of new screening tests.

1.4. Needs for new tests

Molecular testing provides opportunities to increase access to cervical cancer screening in LMICs through enabling accurate

see-and-treat strategies and self-sampling. Health systems in LMICs generally can support limited numbers of patient encounters, so high-sensitivity screening tests, such as an HPV DNA test, allow providers to identify at-risk patients at the time of their first visit. Studies have shown that a single HPV DNA test, coupled with appropriate treatment, can reduce cervical cancer mortality by 50% [68]. Current limitations in HPV DNA tests include high per-test cost, instrumentation cost, infrastructure requirements, and complexity of use. Further, molecular testing makes the possibility of self-testing more realistic, as sample adequacy requirements are much more stringent for cytology than for DNA testing [69].

In addition to new molecular tests for screening, innovations in diagnostic technologies could significantly improve the ability to triage patients with positive screening results in LMICs. A promising area of research is in lower-cost, real-time, optical diagnostics, which could increase access to diagnosis and lower rates of overtreatment. Automated visual analysis using optical diagnostic technologies could overcome some of the current limitations of diagnostic technologies, including equipment cost and the need for highly trained personnel [32].

In the remainder of this article, we discuss advances in molecular tests and optical tests that could increase access to cervical cancer screening and diagnosis in LMICs. The commercialized molecular tests we discuss have been selected because they are in use in LMICs or utilize a technology that could be adapted for use in LMICs. In addition, we discuss promising approaches to in-development molecular tests that are not yet commercialized. The optical tests described in this article include innovations in mobile colposcopy and *in vivo* microscopy, along with computational approaches to improve decision support for users of optical imaging tests.

Finally, we present our views and critical opinions on future directions of technology innovation for cervical cancer screening and diagnosis in LMICs at the conclusion of the article. In short, we see mRNA testing, self-sampling, and real-time optical imaging diagnostics with machine learning playing a role in improving global cervical cancer prevention efforts. The challenges associated with implementing cervical cancer screening in very low-resourced settings, in our opinion, cannot be met with the tools and healthcare worker capacity we currently have. To solve the problem, we have to combine accurate screening, detection, and treatment into a single visit, and we need to strengthen infrastructure. We review what currently available tools exist prior to presenting our views on the future of technologies for cervical cancer screening and diagnosis in low-resource settings.

2. Recent advances in molecular tests

Molecular testing is clinically useful as a first line screening method for cervical cancer. As previously described, HPV testing is often used in conjunction with cytology or as a standalone test for primary cervical cancer screening. In LMICs, HPV testing is a recommended screening practice when sufficient resources are available. Here, we describe commercialized and in-development advances in molecular tests for cervical cancer screening in LMICs.

2.1. Commercialized tests

Several assays for HPV testing in LMICs are commercialized and in routine use. Some of the tests are packaged as assays that require standard laboratory equipment, and others are fully integrated and are sold with all required instrumentation. The tests target different biomarkers, including DNA, RNA, and proteins. In addition, the partial genotyping capability of each test varies. This review focuses on tests that are currently in routine use in low-resource settings, subsidized for certain LMICs, or use a detection method that could be translatable to the point-of-care, such as isothermal amplification. A summary of the tests discussed in this section can be found in Table 1. The selected tests do not include all FDA-approved HPV screening tests. For example, the Roche cobas test is in fairly widespread use in the United States; however, because of the large instrument footprint, reliance on advanced infrastructure, and the presence of an alternative DNA test that we see as more appropriate for use in LMICs, the GeneXpert test, we have not included Roche cobas in our review. Summaries of high-resource commercialized HPV tests [28,70] and their enabling methods [71] have previously been described. We acknowledge the challenges of comparing test parameters, i.e. citing comparable manufacturing cost estimates or performance data across different sites; in this section, we present representative values as cited in the literature.

2.1.1. Hybrid capture tests

Hybrid capture HPV tests rely upon hybridization of target DNA to synthetic RNA. The DNA/RNA hybrids are then detected in enzyme-linked immunosorbent assay (ELISA) format. Hybrid capture approaches generally are less sensitive than amplification methods but detect DNA in the clinically relevant range.

The *digene* HC2 DNA Test (Qiagen) is a hybrid capture assay that relies on standard laboratory equipment and protocols and for which all required reagents for high-risk HPV detection are packaged and sold. The result is a qualitative indicator of the presence of any high-risk HPV types without genotyping. The test is complex and requires significant hands-on time. It is also expensive, at an estimated cost of US$71 per test [79]. The test has high sensitivity and relatively high specificity, though there is some cross-reactivity with low-risk types [80]. One of the biggest challenges with implementing HC2 is the required laboratory infrastructure and instrumentation [44], including a plate reader, shaker, calibrated set of pipettes, and refrigerator.

In an attempt to bring HPV DNA testing closer to the point of care, Qiagen developed careHPV, a test that utilizes the same hybrid capture testing principles as HC2 in a more point-of-care-friendly format. Similarly to HC2, careHPV produces a pooled high-risk result without genotyping. Along with the required reagents, careHPV packages the necessary instruments, which still include a plate reader and orbital shaker. While the single source of all testing equipment is helpful, the total cost of instrumentation is still estimated to exceed US$20,000, and a stable power supply is necessary [81]. In addition, test complexity remains a major challenge with careHPV. The 96-well plate format requires training for users to competently and confidently run the test. The per-test cost can be as low as US$5, but only if a batch of 90 samples are run at a time [30,82]. Additionally, the low per-test cost assumes scale-up to 20,000 tests and that no

Table 1. Summary of selected commercially available HPV tests for cervical cancer screening.

Test	Biomarker detected	Detection method	Partial genotyping?	Limit of Detection	Sensitivity (%)[c]	Specificity (%)[c]	Population	Per-test cost (USD)	Instrument cost (USD)	Sample prep. integrated	Batching
digene HC2 (Qiagen)	DNA	Hybridization, chemi-luminescence	No	100,000 copies/mL [72]	85.7–97.5 [73,97]	81.8–85.4 [73,97]	Screening [97], referral [73]	$71 [79]	– [f]	No	Yes
careHPV (Qiagen)	DNA	Hybridization, chemi-luminescence	No	100,000 copies/mL [84]	85.7–88.1 [48,73]	83.1–83.7 [48,73]	Referral [73], Screening + referral [48]	$5–42 [30,81,82]	$20,000 [81]	No	Yes
GeneXpert HPV (Cepheid)	DNA	qPCR, fluorescence	16, 18/45	2903 to 50,493 copies/mL[d] [74]	94% [75]	83% [75]	Screening + referral [75]	$20 (est.) [90]	$11,530–71,500 [91]	Yes	No
Aptima HPV (Hologic)	RNA	TMA, fluorescence	16, 18/45	60 to 1220 copies/mL[e] [72]	97.5 [97]	90.2 [97]	Screening [97]	$12[a] [98] ($30 [91])	$0[a] [98] ($150,000 [91])	Yes	No
NucliSENS EasyQ (bioMérieux)	RNA	NASBA, fluorescence	16, 18, 31, 33, 45[b]	230 to 30,000 copies/mL [44]	69–79.3 [100–102]	36–72.6 [100–102]	Referral [100–102]	$23 (est.) [91]	$45,000–65,000 [91]	No	Yes
Proofer (PreTect)	RNA	NASBA, fluorescence	16, 18, 31, 33, 45[b]	4000 to 5000 copies/mL [76,77]	78.1 [105]	75.5 [105]	Screening + referral [105]	–[f]	– [f]	No	Yes
OncoE6 (ArborVita)	Protein	Sandwich assay, lateral flow	16, 18[b]	20,000 cells/mL [114]	31.3–53.5 [48]	98.9–99.4 [48]	Screening + referral [48]	– [f]	$2,000 [70]	No	No

qPCR: quantitative polymerase chain reaction; TMA: transcription-mediated amplification; Nucleic acid sequence-based amplification (NASBA)

[a]*subsidized cost in eligible countries under the Hologic Global Access Initiative with unsubsidized costs in parentheses; (est.): estimated test costs based on different assay using same platform*

[b]*only types detected for these tests; the remaining tests produce a pooled high-risk result plus partial genotyping.*

[c]*all reported sensitivities and specificities are compared against a gold standard of biopsy-proven CIN2 +*

[d]*values converted from international units/milliliter (IU/mL) to copies/mL using the WHO International Standard, NIBSC code 06/202* [78]

[e]*values calculated from copies/reaction.*

[f]*to our knowledge, values are not found in the literature.*

additional capital investments will be required. Per-test cost estimates, considering both equipment and supplies, were reported as US$42 in a pilot careHPV implementation program in Myanmar [81]. Batching requirements can lengthen turnaround time in low-throughput clinical settings. The four-hour testing time and batching-related delays mean patients almost always have to come back for a second visit to receive results, which increases the likelihood of losing patients to follow-up [30]. Despite the challenges faced by careHPV, many groups have implemented careHPV and evaluated clinical performance in large-scale studies. For example, in a multi-country study with over 16,000 patients, sensitivity and specificity of careHPV on physician-collected cervical samples were 81.5% and 91.6% and on self-collected vaginal samples were 69.6% and 90.9%, respectively. In comparison, sensitivity and specificity of VIA were 59.8% and 84.2% and cytology were 58.4% and 87.7%, respectively [83]. Other studies have validated the use of careHPV in screening programs in low-resource settings [84–87] and with self-collected samples [36,88].

2.1.2. Polymerase chain reaction (PCR) tests

Nucleic acid amplification tests (NAATs) increase the analytical sensitivity and specificity of DNA testing over hybridization approaches, but also generally increase the instrumentation requirements. Hybridization approaches target DNA/RNA hybrids, so there is greater cross-reactivity with other non-targeted genotypes compared with amplification approaches [89]. Polymerase chain reaction (PCR) is the gold standard NAAT and utilizes thermocycling to amplify DNA to detectable levels. Thermocycling generally requires complex instrumentation, increasing the cost of the technologies. However, some PCR approaches, such as the GeneXpert tests from Cepheid (Sunnyvale, CA), limit user steps in a format that is appropriate for use in LMICs.

The GeneXpert HPV Assay is compatible with the GeneXpert family of benchtop analyzers already in global use for tuberculosis (TB) and human immunodeficiency virus (HIV) detection, which could facilitate implementation of HPV testing using the GeneXpert platform. GeneXpert analyzers automate sample preparation, nucleic acid amplification, and fluorescent detection using real-time PCR, providing results with high analytic sensitivity and specificity. The GeneXpert HPV assay detects all high-risk HPV types along with partial genotyping of HPV16 and HPV18/45. The assay requires very little hands-on time and produces a result in an hour [30]. The cassette format is user-friendly, and HPV testing can be rapidly scaled with delivery of HPV testing cassettes in locations that already use GeneXpert analyzers for TB and HIV testing. A major challenge with GeneXpert, however, is the per-test cost. While cost estimates are not yet available for the HPV assay, estimates for the MTB/RIF assay exceed US$20 per test [90], and estimates for the HIV and hepatitis C (HCV) assays range from US$16.80–17.95 per test [91]. The instrument costs range from US$11,530 for a two-module, desktop-based instrument to US$71,500 for a 16-module, laptop-based instrument [91]. The GeneXpert HPV Assay has been validated in large-scale clinical studies with findings that suggest accuracy and reproducibility on-par with well-established HPV assays. Various studies on different patient populations have shown 89–100% sensitivity and 42.6–83% specificity [70,92–94], comparable performance between Xpert HPV, Roche cobas, and *digene* HC2 testing [93,94], and excellent agreement between self-collected vaginal specimens and clinician-collected cervical specimens [95]. If the per-test cost and the infrastructure requirements of the instrument could be reduced, the Xpert HPV assay would be a high priority technology to implement for primary HPV screening.

2.1.3. Isothermal nucleic acid amplification tests

In an effort to reduce the infrastructure requirements of PCR, several isothermal nucleic acid amplification technologies have been developed as previously described [96]. In isothermal approaches, additional enzymes are added to amplification reactions such that all amplification processes can occur at a single temperature. Therefore, simple, single-temperature heaters can be used in place of thermocyclers, reducing infrastructure needs for instrumentation. In this section, isothermal NAATs for RNA will be discussed, though these technologies can similarly be used for DNA amplification and detection.

Hologic (Marlborough, MA) has commercialized two Aptima HPV assays: one that detects all high-risk types without genotyping (Aptima HPV Assay) and one that differentiates genotype 16 from 18 and 45 (Aptima HPV Genotype Assay). The Aptima test utilizes transcription-mediated amplification (TMA) and incorporates automated sample preparation. The clinical sensitivity and specificity have been reported as 97.5% and 90.2%, respectively [97]. The biggest barriers to implementation of the Aptima test are the cost and infrastructure requirements of the instrument that runs the Aptima test, the Hologic Panther [79]. The estimated unsubsidized per-test cost is US$30, and the Panther instrument cost is US$150,000–175,000 [91]. In 2018, Hologic announced a subsidy for tests using its Panther system called the Hologic Global Access Initiative, which is a partnership with the Clinton Health Access Initiative, Inc. and MedAccess (backed by the UK government). The Global Access Initiative ensures a ceiling price of $12 with no requirements for capital expenditure. The $12 per test cost covers 'all necessary reagents and consumables, instrument placement, service and maintenance, freight and logistics, and replacement tests' [98].

Another laboratory-based HPV mRNA test is available in the bioMérieux NucliSENS EasyQ test. The NucliSENS EasyQ detects five high-risk types (16, 18, 31, 33, 45) with high analytical specificity and reported limits of detection ranging from 230 to 30,000 copies/mL for each type [99]. The NucliSENS amplifies target RNA by nucleic acid sequence-based amplification (NASBA) and detects fluorescence through the use of molecular beacons [91]. Studies have shown clinical sensitivities and specificities of 79.3% and 72.6% [100], 69% and 36% [101], and 76% and 63% [102], all in populations of women with abnormal Pap results. The NucliSENS EasyQ instrument cost is estimated at US$45,000, and the accompanying miniMAG instrumentation used for low-volume RNA extractions is estimated at US$20,000. The per-test cost for a comparable assay, the NucliSENS EasyQ HIV test, is US $23 [91]. Pre-processing of the sample, including the method of RNA extraction, has been shown to be important for HPV RNA test accuracy; the NucliSENS extraction method allows for higher sensitivity than other common methods [103]. The sensitivity

may be further improved by employing alternative amplification methods and by incorporating additional genotypes.

Similarly, to NucliSENS EasyQ, the Pretect Proofer mRNA test detects types 16, 18, 31, 33, and 45. Amplification of the five detected genotypes occurs through NASBA, and amplicons are detected in real time by fluorescence monitoring on a plate reader. The test format is a pre-loaded microtiter plate, to which pre-processed samples are added; therefore, the test must be run within a laboratory setting. Thirty samples are run at a time, and the test claims a low hands-on time [104]. The test has a reported clinical sensitivity of 78.1% and specificity of 75.5% among a referral population [105]. Similarly to NucliSens EasyQ, the sensitivity of the Pretect Proofer can be improved with the addition of more high-risk genotypes. Currently, the high specificity of RNA testing might be most useful in combination with DNA testing. If the threshold for positivity of RNA testing is raised, lowering the analytic sensitivity and likely improving the clinical specificity, RNA testing might be most effective when used in combination with high-sensitivity DNA tests.

2.1.4. Protein tests

In comparison with HPV DNA tests, oncoprotein tests generally have lower sensitivity and higher specificity. Arbor Vita (Fremont, CA) has commercialized a lateral-flow based E6 oncoprotein test, OncoE6, for HPV types 16, 18, and 45 [48]. The lateral flow readout is point-of-care-friendly and has separate detection lines for each HPV type, allowing for partial genotyping [49]. Reported clinical sensitivities and specificities of the OncoE6 test range from 31.3% to 53.5% and 98.9% to 99.4%, respectively [48]. When restricting analysis to patients positive for the three genotypes covered by the test, the sensitivity increased to 64.5%; therefore, sensitivity limitations are not solely attributable to missed genotypes [106]. Equipment for the OncoE6 test is fairly low-cost at an estimated US$2,000. However, the test requires over 45 minutes of sample preparation with several pipetting and centrifugation steps, and therefore is not yet an optimal solution for low-resource settings [49,70,106]. Automating sample preparation and limiting hands-on testing time, as well as increasing the number of genotypes detected, could improve the performance and usability of the OncoE6 test.

2.2. HPV tests in development

While careHPV and GeneXpert have had some success in low-resource environments, their cost and infrastructure requirements limit their potential for large-scale screening. Similarly, Aptima use will likely increase with their Global Access Initiative but will be limited by infrastructure requirements of the Panther instrument. To increase accessibility, several promising technologies are being developed to reduce the cost and infrastructure necessary for HPV molecular testing (Table 2, Figure 3).

2.2.1. PCR tests

Global Good has partnered with medical device developer QuantuMDx (Newcastle upon Tyne, UK) to develop an HPV assay using the QuantuMDx platform, Q-POC. The Q-POC analyzer is being developed as a platform for a wide variety of clinical applications [108]. The Q-POC process includes a simple specimen processing protocol, in which the swab is transferred to a collection tube, the swab handle is removed, the cap to the collection tube is closed, and the tube can be directly connected to the assay cassette for elution. Next, PCR amplification occurs within the Q-POC instrument. For HPV, this includes a single multiplex reaction, amplifying 13 high-risk types. Detection occurs on-board in array format; specifically, an array of DNA probes captures amplified target DNA for sequence-specific, fluorescent detection. The Q-POC platform reportedly has a small benchtop footprint, requires less than 20 minutes inclusive of sample preparation and detection, is battery powered with the capability to perform 15 tests per day, and has a limit of detection of 10 to 50 copies per reaction for each high-risk type. The probes have high specificity relative to other high- and low-risk HPV types, enabling genotyping, and high specificity relative to other sexually transmitted viruses [109]. When produced at scale, the estimated manufacturing cost for the Q-POC analyzer is GB£500 (approximately US$650) and for each cartridge ranges from GB £5–20 (approximately US$6.50–26) based on test complexity [108]. The Q-POC test is undergoing a multi-site clinical evaluation after a preliminary pilot study under ideal laboratory conditions. The strengths of the test are ease of sample preparation, low time-to-result, and reportedly high sensitivity and specificity. Results of the ongoing clinical evaluation will be important in assessing the potential for scale-up and widespread use.

2.2.2. Isothermal nucleic acid amplification tests (naats)

As previously described, isothermal NAATs can reduce infrastructure requirements associated with thermocycling approaches, and therefore are promising options for use in LMICs. Here, an approach toward HPV isothermal NAAT development and a promising integrated isothermal NAAT platform that could be expanded to an HPV application are described (Figure 3(a–b)).

The Klapperich lab at Boston University has developed a paper-based system for extraction, amplification, and detection of HPV 16 DNA [110]. The system uses isothermal loop-mediated amplification (LAMP) and lateral flow detection to identify as low as 10^4 copies of HPV16 DNA from samples in less than one hour. The test is made from paper and plastic and requires only a single-temperature heater (63°C) to run, reducing the need for complex infrastructure or expensive equipment. However, the test does involve a significant number of user steps, making it difficult to implement without trained personnel in low-resource settings. To date, 10 clinical samples have been run on the paperfluidic chip, and while the sensitivity looked promising (5/5 positive HPV+ samples), the test produced false positives compared to qPCR (3/5 negative HPV- samples). The Klapperich lab suggests spurious LAMP primer interactions might be responsible for the false positive results, and their future work involves addressing these primer interactions as well as making the device more user-friendly. In addition, the device detects HPV 16 alone and would need to be expanded to additional HPV types for complete utility as a screening tool, Nevertheless, the paper and plastic format, reduced infrastructure

Table 2. Summary of in-development HPV tests for cervical cancer screening.

Test	Biomarker detected	Detection method	Partial genotyping?	Limit of Detection	Sample preparation integrated?	Phase of Development
Q-POC (QuantuMDx)	DNA	PCR, fuorescence	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68a, 68b	10 to 50 copies/ reaction [109]	yes	Pilot study under ideal conditions (n = 70; concordance analysis in progress) [109]; pursuing multi-site clinical evaluation [107]
Paperfluidic chip (Klapperich lab)	DNA	LAMP, lateral flow assay	16	100,000 copies/ mL [110]	No	Addressing limitations of current prototype after initial pilot test (n = 10 in ideal conditions) [110]
Onco E6/E7 Eight HPV Type Test (Arbor Vita)	Protein	Sandwich assay, lateral flow	16, 18, 31, 33, 35, 45, 52, 58	20,000 to 100,000 cells/mL [114]	No	Pilot study completed under ideal conditions with laboratory technicians (n = 259, 31 CIN2+; Se: 67.7%, Sp: 89.5%) [115]; currently pursuing larger-scale validation study

PCR: polymerase chain reaction; LAMP: loop-mediated isothermal amplification

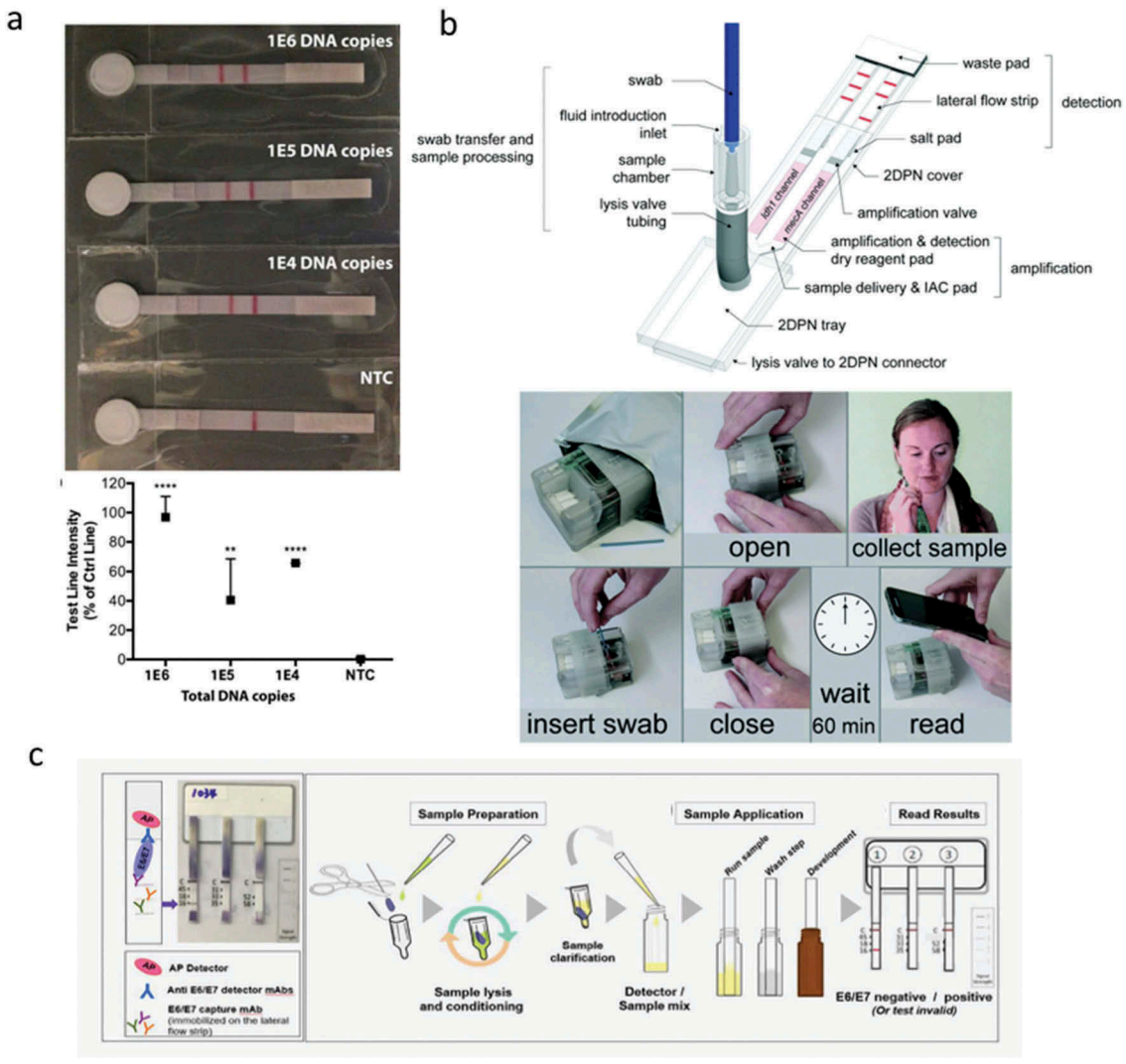

Figure 3. In-development paperfluidic HPV tests. (a) Paperfluidic test developed by Rodriguez et al. Example results with input DNA copies ranging from 1E4 to 1E6 and a no-target control (NTC) are shown. Test line intensities indicate positive signals were formed at the test line in the presence of DNA over 1E4 total input copies of HPV 16 DNA; no test line signal formed with the NTC condition, indicating analytic specificity of the test. (b) MAD NAAT test developed by LaFleur et al. Internal components, including sample inlet port, lysis chamber, sample elution mechanisms, amplification reagents, and lateral flow detection are shown (top). The method of use is also shown (bottom), with 5 user steps spanning roughly an hour. (c) OncoE6 8-type test developed by Zhao et al. Left: example lateral flow strips and capture chemistries are shown. Capture antibodies are embedded in lateral flow strips and capture E6/E7 proteins. Detection antibodies form a sandwich assay with the immobilized proteins, and alkaline phosphatase (AP) binds and produces a purple colorimetric signal. Three total test strips are used to detect 8 high-risk types. Right: method of use of OncoE6 8-type test is shown. First, a cervical swab is placed in buffer, and the sample is lysed and conditioned. Next the sample is added to detection buffer, and lateral flow strips are placed into the mixture. The lateral flow strips are then moved into different buffers for washing and signal development. Finally, test strips are read by ensuring control lines are positive on all strips and identifying colorimetric signals at any of the test lines. In this figure, the sample is positive for type 16. Figure 3(a,b) reproduced from [110] and [111], respectively, with permission of the Royal Society of Chemistry from; permission conveyed through Copyright Clearance Center, Inc. Figure 3(c) reproduced from [106] with permission of John Wiley and Sons.

requirements, and on-device sample preparation are promising steps toward a truly point-of-care HPV DNA test.

Additionally, several groups have developed microfluidic or paper-based platforms for nucleic acid amplification, which could be expanded to low-cost HPV molecular testing. The Yager lab at the University of Washington developed the multiplexed autonomous disposable nucleic acid amplification test (MAD NAAT), which is a sample-to-answer nucleic acid amplification test for use in low-resource settings [111]. MAD NAAT uses feedback-controlled heaters, two wax valves, dried reagents, and on-device buffer storage to 1) lyse and fragment DNA, 2) amplify DNA, and 3) release amplicons onto a lateral flow strip for detection without any user input. The on-device heater is used in both sample preparation (95°C for 10 minutes) and isotheral amplification (50°C for 30 minutes), as well as to melt the wax valves to release DNA into the amplification chamber and lateral flow strip. The strengths of the device are its short time-to-result (<2 hours), potential low cost, and integrated design that limits user steps and the potential for sample contamination. The test was designed for amplification of bacterial DNA from a nasal swab, not viral HPV DNA from a cervical swab, and it still needs to be optimized for use with clinical samples and for repeatability. As last reported, the completion rate of the device was 62%, with device failures due to hardware and

flow issues. Nevertheless, the platform is a promising step toward a point-of-care molecular test and could potentially be translated to HPV DNA detection.

Other groups have developed paper platforms for individual components of nucleic acid testing including sample preparation, amplification, and detection, which are discussed in a recent review article [112]. Many of these devices use isothermal amplification of DNA with a single temperature heater, or body heat in the case of recombinase polymerase amplification (RPA), to reduce equipment and infrastructure needs [113]. Despite these advances, no truly point-of-care platform for DNA or RNA amplification has been validated with large-scale clinical studies in low-resource settings.

2.2.3. Protein tests

Arbor Vita, in collaboration with PATH, has recently developed a new prototype of their OncoE6 test (Figure 3(c)). The prototype, the Onco E6/E7 Eight HPV Type Test, expands detection to both E6 and E7 oncoproteins and includes two additional lateral flow strips for oncoprotein detection of HPV types 16, 18, 31, 33, 35, 45, 52, and 58 [114,115]. The test works in a similar method to the OncoE6, with individual test lines for genotyping and with complicated and user-intensive sample preparation. In a small clinical study (n = 259), the new prototype had a sensitivity and specificity of 67.7% and 89.5%, respectively [115]. To evaluate true sensitivity and specificity, larger studies on broader populations will need to be conducted. The increased test sensitivity relative to the three-type test is likely due to the increased number of HPV types tested. However, the sample preparation and sensitivity limitations of the OncoE6 test remain the same with this prototype. This test will need to be further evaluated clinically to understand its potential role in screening and triage.

3. Recent advances in optical tests

Following molecular screening, confirmatory diagnosis is often performed with optical imaging methods, e.g. colposcopy. Traditional optical confirmation technologies, like the colposcope, are often unavailable in LMICs due to their reliance on highly trained personnel and costly equipment. However, new approaches toward lower-cost imaging technologies with decision support could increase access to optical confirmation of positive screening results in LMICs. Optical imaging technologies discussed here include innovations in mobile colposcopy, *in vivo* microscopy, and image analysis methods for automated decision support.

3.1. Mobile colposcopy

Several groups have developed mobile colposcopes in order to reduce the cost associated with traditional colposcopes. The technologies described here are at different stages of commercialization and are all being evaluated clinically. The devices and representative cervical images taken with each device are shown in Figure 4.

3.1.1. Mobileodt

MobileODT (Tel Aviv, Israel) has developed a commercial mobile colposcope, the Enhanced Visual Assessment (EVA) Colpo, to lower the cost of and complexity of colposcopy. The device consists of an Android smartphone outfitted with magnifying optics and a set of battery-powered LEDs for illumination. Additionally, MobileODT has developed a mobile app allowing providers to store patient information, acquire and store cervical images, and record locations of biopsies and other clinical observations. The MobileODT system can also facilitate remote

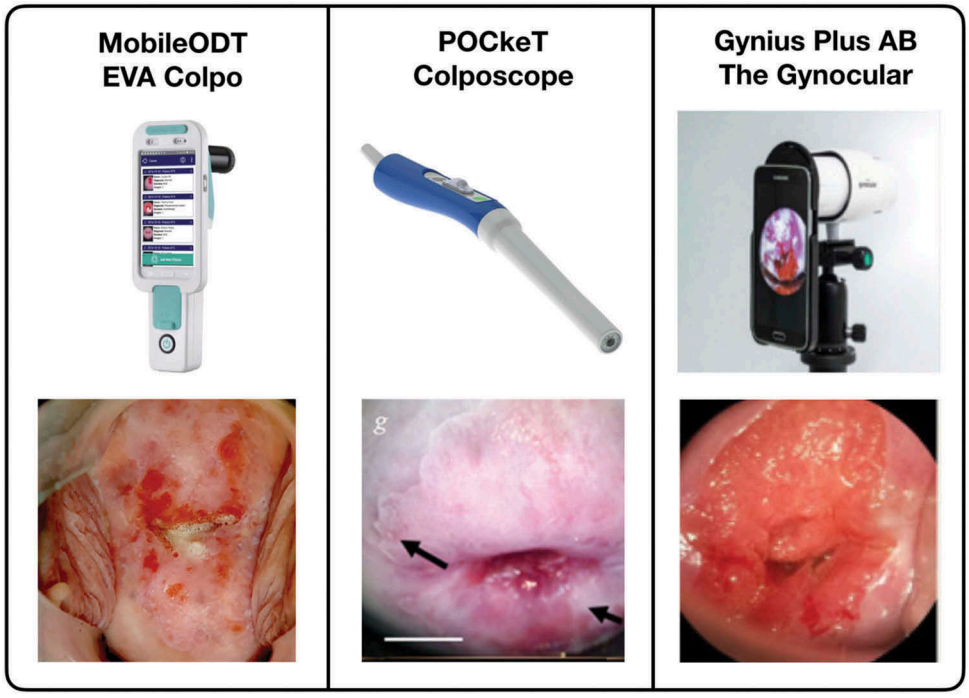

Figure 4. Example low-cost mobile colposcopy systems. For each mobile colposcope (top), example cervical images taken with that device are shown (bottom). Reproduced from [117] with permission (MobileODT) [118], with permission of Wolters-Kluwer Health, Inc. (POCkeT Colposcope), and [124] with permission (The Gynocular) .

consultation through live-streaming over telecommunications networks, which can potentially increase access to expert colposcopy in medically underserved areas.

Initial clinical validations of the MobileODT EVA Colpo system have been promising, though a large scale validation study has yet to be reported. In a small study in California, the EVA Colpo was shown to reduce both false positive and false negative rates when used in conjunction with Pap testing [116]. In a study involving 250 HIV+ and HIV- women in Cambodia, providers' impressions of digital colposcopy performed with the EVA Colpo system was consistent with pathology in all 30 women who underwent confirmatory biopsies. The study concludes that digital colposcopy was effective in differentiating CIN1 from CIN2+, and the proposed testing algorithm moving forward in Cambodia should be HPV testing on self-collected samples, confirmatory testing with digital colposcopy, and treatment on all positive colposcopy results [117].

3.1.2. Pocket colposcope

The Point of Care Tampon-based digital (POCkeT) Colposcope was developed by researchers at Duke University to be ultra-portable and low cost. Whereas traditional colposcopes are designed to acquire images at a distance, the POCkeT colposcope is designed to image the cervix from within the vaginal canal (only a few centimeters from the cervix). Reportedly, the materials cost of the POCkeT colposcope is roughly US$500, compared with US$20,000 for a standard-of-care colposcope [118]. In addition, the POCkeT Colposcope can be used with an insertion device that is much smaller than a conventional speculum, potentially reducing barriers to use that are associated with fear of discomfort or embarrassment [119].

In initial studies, the POCkeT Colposcope showed comparable performance to commercially available colposcopes in terms of resolving power, color reproduction accuracy, lens distortion, and illumination [120]. Earliest designs of the POCkeT Colposcope faced challenges with specular reflection, illumination uniformity, and fogging effects; however, subsequent designs have improved each of these areas [121]. A small-scale clinical evaluation of the POCkeT colposcope demonstrated fair agreement in physician interpretation of images acquired with both standard and POCkeT colposcopes [118].

3.1.3. The gynocular

Gynius Plus AB (Göteborg, Sweden) has developed a compact monocular colposcope, called the Gynocular, which has comparable optical performance to standard-of-care colposcopes, contains a battery-powered illumination system, and weighs just under 0.5 kg [122]. Practitioners can visualize the cervix directly by looking in an eyepiece on the Gynocular, or digital images can be acquired by attaching a mobile device camera to the eyepiece. The Gynocular has been used in conjunction with the Swede score (a standardized scoring system for colposcopy findings) to conduct clinical evaluations of the device in Sweden, Bangladesh, and India [122–124]. In India, digital Gynocular images from 94 VIA-positive women were evaluated remotely by six colposcopists using the Swede score system. Diagnostic predictions from the remote evaluation were found to be on par with predictions from live colposcopy evaluation (area under curve 0.71 vs 0.69) for detection of CIN2+ lesions.

3.2. *In vivo* microscopy

In addition to mobile colposcopy, a promising area of imaging research for cervical cancer applications is *in vivo* microscopy, which affords greater resolution. Several optical approaches toward *in vivo* microscopy for both low- and high-resource settings are described here.

3.2.1. High-resolution microendoscope (HRME)

The high-resolution microendoscope (HRME) is a fiber-optic fluorescence microscope that can be used to detect cellular and subcellular features *in vivo*, thereby enabling assessment of suspected precancerous and cancerous cervical tissue. It is used in combination with the fluorescent dye proflavine, which is applied topically. During imaging, the flexible HRME probe is placed in contact with the cervix, enabling the clinician to view the size, shape, and distribution of epithelial cell nuclei in real time. The ability to detect subcellular features in real time with the HRME can help the clinician make a clinical diagnosis, can help guide biopsy site selection, and in some settings where histopathology is unavailable or impractical, can enable see-and-treat strategies. The HRME also provides an opportunity for use by non-specialist providers by generating an automated real-time diagnosis.

In small-scale clinical studies, the HRME has shown high diagnostic accuracy in differentiating CIN2+ from non-neoplastic tissue. In an early study of 52 women in Botswana, the HRME showed a sensitivity of 86% and a specificity of 87% compared with histopathology [125]. In a subsequent study of 59 women in Brazil, a sensitivity and specificity of 92% and 77%, respectively were reported [126]. More recently, a cluster-randomized community trial used the HRME in a mobile van to reach women for diagnostic follow-up in rural areas of Brazil. Rates of follow-up among 144 women included in the study increased from 64% in the standard of care, i.e. women had to travel to a central hospital for diagnostic follow-up, to 87% in the mobile van that traveled to their community. Furthermore, the diagnostic performance of HRME was comparable to colposcopy [127]. Larger-scale studies are currently underway in El Salvador, Brazil, and the Rio Grande Valley along the Texas-Mexico border.

Recent improvements in HRME instrumentation have further reduced cost while preserving image quality by leveraging mobile device hardware and single board computers (Figure 5) [128–130]. These even lower-cost designs can further improve uptake of HRME in resource-constrained settings. Additionally, newer optical designs that have demonstrated improved axial resolution and contrast through optical sectioning are under development [131,132]. Studies to assess whether improved optical sectioning for HRME systems can improve detection of neoplastic tissues are underway.

3.2.2. Other *in vivo* microscopy technologies

In addition to HRME, other existing technologies have demonstrated promising results toward *in vivo* assessment of cervical tissue. Recently reported studies assessing *ex vivo* cervical specimens using probe-based confocal laser endomicroscopy [133–135] and optical coherence tomography or microscopy [136–

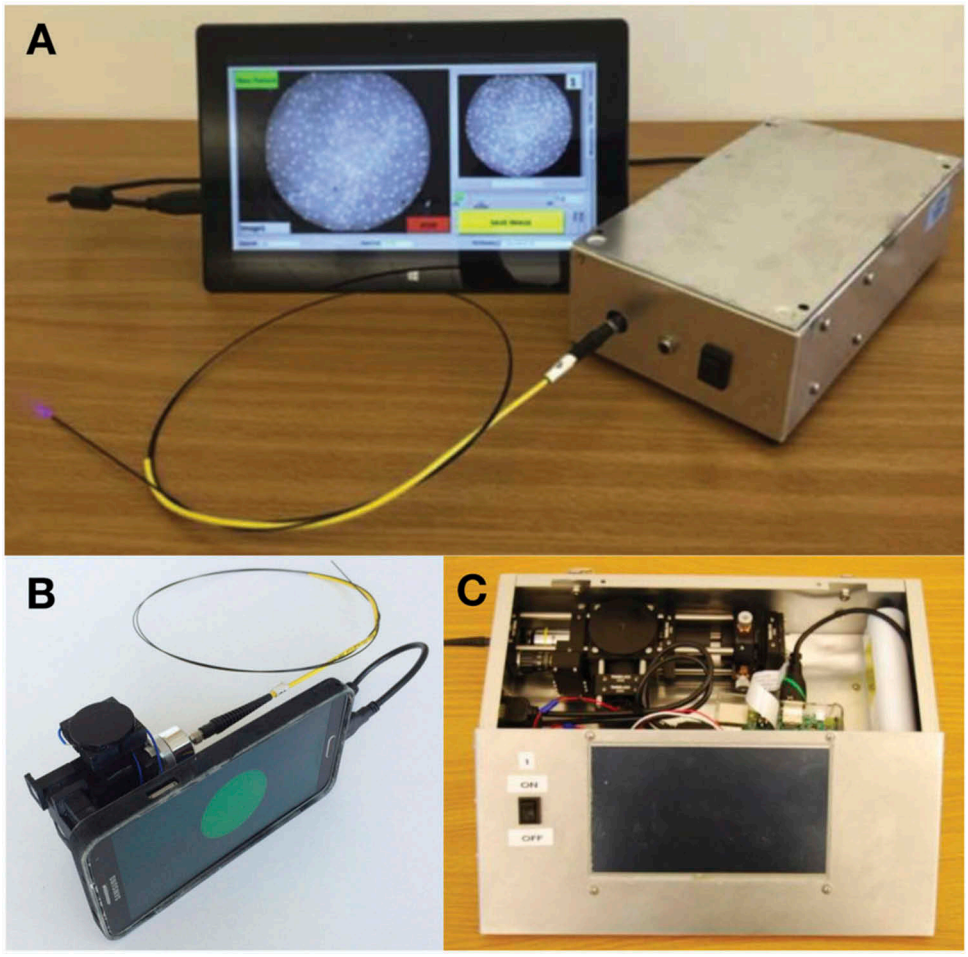

Figure 5. Example images of low-cost high-resolution microendoscope (HRME) systems. (a) The tablet HRME with example images and user interface, (b) mobile phone HRME, and (c) Raspberry Pi HRME systems are shown. In all systems, a flexible fiber-optic probe is used. (a-b) Reproduced from [128,129] with permission. (c) Reproduced from [130] with permission from Elsevier.

138] suggest these methods have potential for distinguishing neoplastic cervical tissues. As yet, these methods have not been validated extensively for cervical imaging *in vivo*, and the high cost of these instruments is likely to be a barrier to their implementation in LMICs.

3.3. Decision support

Placing lower-cost technology into the hands of practitioners is a step forward in terms of accessibility, but confidence in the technology and removing subjectivity to reduce inter-user variability is important for proper uptake and consistent performance. Improved algorithms for real-time image classification can bolster confidence in diagnostic decision-making. One approach to improve image classification algorithms is through the use of machine learning (ML) or artificial intelligence (AI). AI has been evaluated with cervicography, a procedure in which an image of the cervix is taken with a fixed-focus camera during screening. In a study including 9,406 women, real-time interpretation of cervigrams without decision support yielded an area under the curve (AUC) of 0.69; after the fact, when AI-enabled image classification was developed, the reported AUC was 0.91, illustrating the potential for AI-enabled decision support [32]. Similarly, cervigrams obtained using VILI and the POCkeT Colposcope were trained using a support vector machine (SVM) classifier, and the resulting algorithm produced a sensitivity of 89.2%, a specificity of 66.7%, and an AUC of 0.84. Decision support on a procedure as variable as VILI would be helpful in improving reproducibility and diagnostic performance [139].

After-the-fact processing of images is distinct from real-time image classification, which will be required for clinically useful decision support. MobileODT has announced a collaboration with the National Cancer Institute to implement AI image classification into their system. Clinical performance of this approach has yet to be evaluated. Guidelines and approaches to artificial intelligence for colposcopic image classification have previously been discussed [140].

4. Conclusion

Despite the increasing burden of cervical cancer incidence and deaths in LMICs, screening for cervical cancer in low-resource settings remains limited by cost, equipment, and complexity. Commercially available HPV DNA tests such as careHPV and GeneXpert are being used to screen women in LMICs; however, the per-test cost and infrastructure requirements limit their sustainability and scalability for country-wide screening. Recent advances toward point-of-care molecular testing, including

paper-based approaches and emerging technologies like the QuantuMDx Q-POC test, hold promise; however, these technologies still need to be evaluated for clinical use. These innovations help bring molecular testing closer to the point of care, so that screen-and-treat options can be effectively implemented in LMICs.

Optical diagnostic tests are important for determining who needs treatment among those who screen positive. Without accessible diagnostic tests, screen-and-treat programs are implemented and lead to high rates of overtreatment [40]. Several low-cost optical diagnostic tests are in development and undergoing clinical evaluation. Additionally, applying machine learning to diagnostic algorithms can improve decision support for non-specialist providers, making the use of optical diagnostic tests more likely to succeed widely in low-resource settings.

5. Expert opinion

We need a set of technologies that enable accurate assessment of cervical precancer and cancer within a single visit (<1 hour) without expensive instrumentation and with a low per-test cost. Clinically, HPV DNA is the best screening option we have, but currently available technologies remain too expensive and too complex to effectively scale.

Within five years, we expect that commercial entities and academic institutions will be closer to developing a point-of-care HPV DNA test for screening in low-resource areas. Unless the per-test cost of commercial HPV DNA tests like GeneXpert decreases, it does not seem likely that scale-up for country-wide screening in LMICs will be sustainable. However, increased usage of GeneXpert machines already implemented in TB and HIV programs will likely be leveraged to the benefit of cervical cancer screening programs. In five years, promising technologies like the QuantuMDx Q-POC test may be closer to implementation, depending on the per-test and instrument costs. However, we expect a paper-based HPV DNA test to be developed and in clinical testing within two years. If a paper-based screening test can produce accurate clinical results, we see high potential for scale-up.

An example of a major success in the field of point-of-care testing is the human immunodeficiency virus (HIV) rapid screening test. The format, subsidized cost, and time-to-result of HIV rapid tests allow for screening to happen nearly anywhere. Major challenges that remain with HIV screening include stigma and lack of interaction with medical systems, presenting obstacles to reaching key populations. In other words, the technology is no longer the limiting factor with HIV screening. Until a format similar to the HIV rapid screening test and sustainable financing for scale-up are achieved on a truly point-of-care HPV DNA test, HPV DNA testing will remain possible only in more centralized facilities with sufficient laboratory infrastructure. As the technology improves, we hope to see more programs that are able to screen people who do not have as much contact with medical systems. In particular, harder-to-reach populations who have been underserved in terms of cervical cancer screening include people living in rural and/or medically underserved areas, undocumented immigrants, women who are homeless or incarcerated and transgender men. While researchers have initiated promising programs to reach out to women who are homeless [141] and transgender men [142], we anticipate greater ability to screen and follow-up with patients with fewer technology barriers. Self-collection of samples with high-performing diagnostics will especially increase ability to screen people who are harder to reach [39].

While there are many promising approaches to achieving an ideal HPV test, we see the most potential in approaches that limit instrumentation and minimize hands-on time. High instrumentation requirements often come from robotic sample manipulation and thermocycling used in traditional amplification approaches. Paper-based test designs are a feasible approach to circumventing the need for robotic sample manipulation. The format of the tests reported by Rodriguez *et al*. and LaFleur *et al*. could lower per-test cost while largely removing the need for instrumentation [110,111]. Additional paper-based testing approaches should be developed in an attempt to lower cost and instrumentation requirements. Traditional thermocycling approaches can also increase instrumentation complexity, and therefore cost and difficulty with maintenance and repair. In comparison, isothermal amplification methods like recombinase polymerase amplification (RPA) or loop-mediated isothermal amplification (LAMP) only require a single-temperature heater. RPA, in particular, has a low limit of detection of fewer than 10 total copies, and can be incubated at 37°C, the lowest temperature of available isothermal amplification approaches. An integrated test that utilizes a paper-based platform for sample manipulation and detects through either hybridization or amplification approaches could lead to a lower-cost test with lower complexity.

Sample preparation can limit the utility of otherwise well-performing tests. The sample preparation approach employed by the QuantuMDx Q-POC test, if proven to enable high-sensitivity detection, could provide a good model for cervical swab-based sample processing. In this approach, the swab is immediately placed into a sample tube, the handle of the swab is removed, the tube top is closed, and the tube is attached to the testing cassette for elution.

With a point-of-care HPV DNA screening test, a screen-and-treat program could be effectively implemented, although patients may be overtreated without effective diagnostic technologies. The recent developments of low-cost optical systems like the HRME with automated image analysis or cervicography with AI may help to reduce overtreatment if used in triage with a screening test.

We believe that within five years lower cost technologies like the mobile ODT, POCkeT Colposcope, or HRME will have more widespread use in centralized sites or in mobile vans for improved cervical cancer diagnosis. However, we see the best diagnostic option for a low-resource area as cervical imaging with real-time, AI-enabled decision support, which would reduce the need for equipment other than a camera or mobile phone while providing increased specificity for precancerous and cancerous lesions. Studies assessing the accuracy of AI-enabled decision support tools will be crucial to validate the utility for diagnostics. With more powerful mobile phone processing systems, it is likely AI-enabled algorithms will be deployed on mobile phone platforms within the next five years. If AI decision support can be accessed in real time with a mobile phone and clinically useful accuracy is well established, diagnosis of cervical precancerous and cancerous

lesions will become more accessible to patients in low-resource settings. Other diagnostic methods that rely on clinical interpretation will remain possible only in more centralized areas with highly trained providers.

We also envision a possible path forward that relies more heavily on HPV mRNA testing. In scenarios in which only one test might be feasible, quantitative mRNA testing may provide the highest quality clinical information. Qualitative mRNA tests with thresholds set for high specificity are less sensitive than DNA testing, and therefore require repeated testing; lower thresholds for qualitative mRNA tests lead to similar sensitivities and specificities as DNA tests. Clinical utility of a single, quantitative mRNA test will need to be validated, as a single mRNA measurement is not necessarily predictive of disease progression. However, because disease progression requires sustained overexpression of mRNA, testing for HPV mRNA may allow for more accurate assessment of progression. Sample preparation, RNA preservation, testing costs, and instrumentation requirements are currently barriers to widespread mRNA testing. We see mRNA test development as a worthwhile pursuit that could potentially change cervical cancer screening program algorithms globally.

In addition to innovations in molecular and optical technologies alone, we see an opportunity to combine information from molecular and optical tests to improve diagnosis. With self-collected HPV DNA swabs, a truly point-of-care screening test, and lower cost optical diagnostics with real-time decision support, a single visit including accurate screening, diagnosis, and treatment can become the new standard of care in LMICs.

In summary, we see opportunities for technology to improve access to cervical cancer screening among harder to reach populations. Innovations in molecular screening and optical diagnostics could allow for accurate, affordable screen-and-treat methods to detect and treat cervical precancer in a single visit. Developing and translating low-cost, easy-to-use, clinically-relevant molecular and optical testing technologies could reduce the burden of cervical cancer globally.

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Declaration of interest

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394–424.
2. Cervical cancer [Internet]. World Health Organization; 2019 [cited 2018 Apr 6]. Available from: http://www.who.int/cancer/prevention/diagnosis-screening/cervical-cancer/en/
3. Singh GK, Azuine RE, Siahpush M. Global inequalities in cervical cancer incidence and mortality are linked to deprivation, low socioeconomic status, and human development. Int J MCH AIDS. 2012;1:17–30.
4. Wang C, Wright TC, Denny L, et al. Rapid rise in detection of human papillomavirus (HPV) infection soon after incident HIV infection among South African women. J Infect Dis. 2011;203:479–486.
5. World Health Organization. Comprehensive cervical cancer control: a guide to essential practice. 2nd ed. Geneva, Switzerland: World Health Organization; 2014.
6. Cervical Cancer Action. Progress in cervical cancer prevention: the CCA report card; 2012.
7. Bruni L, Diaz M, Barrionuevo-Rosas L, et al. Global estimates of human papillomavirus vaccination coverage by region and income level: a pooled analysis. Lancet Glob Heal. 2016;4:e453–e463.
8. Carcangiu ML, Kurman RJ, Carcangiu ML, et al., editors. WHO classification of tumours of female reproductive organs. 4th ed. Lyon, France: International Agency for Research on Cancer; 2014.
9. Castle PE, Stoler MH, Wright TC, et al. Performance of carcinogenic human papillomavirus (HPV) testing and HPV16 or HPV18 genotyping for cervical cancer screening of women aged 25 years and older: a subanalysis of the ATHENA study. Lancet Oncol. 2011;12:880–890.
10. Cuzick J, Clavel C, Petry K-U, et al. Overview of the European and North American studies on HPV testing in primary cervical cancer screening. Int J Cancer. 2006;119:1095–1101.
11. Mayrand M-H, Duarte-Franco E, Rodrigues I, et al. Human papillomavirus DNA versus Papanicolaou screening tests for cervical cancer. N Engl J Med. 2007;357:1579–1588.
12. Nanda K, McCrory DC, Myers ER, et al. Accuracy of the Papanicolaou test in screening for and follow-up of cervical cytologic abnormalities: a systematic review. Ann Intern Med. 2000;132:810–819.
13. Stoler MH, Schiffman M. Interobserver reproducibility of cervical cytologic and histologic interpretations: realistic estimates from the ASCUS-LSIL triage study. JAMA. 2001;285:1500.
14. Safaeian M, Solomon D, Castle PE. Cervical cancer prevention—cervical screening: science in evolution. Obstet Gynecol Clin North Am. 2007;34:739–760.
15. Mustafa RA, Santesso N, Khatib R, et al. Systematic reviews and meta-analyses of the accuracy of HPV tests, visual inspection with acetic acid, cytology, and colposcopy. Int J Gynecol Obstet. 2016;132:259–265.
16. Curry SJ, Krist AH, Owens DK, et al. Screening for cervical cancer. JAMA. 2018;320:674.
17. Massad LS, Einstein MH, Huh WK, et al. 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. J Low Genit Tract Dis. 2013;17:S1–S27.
18. Jeronimo J, Castle PE, Temin S, et al. Secondary prevention of cervical cancer: ASCO resource-stratified clinical practice guideline. J Glob Oncol. 2017;3:635–657.
• **Describes guidelines on primary HPV screening and management for four tiers of resource levels globally.**
19. RIVM. Cervical cancer screening programme [Internet]; 2011 [cited 2019 Jun 9]. Available from: https://www.rivm.nl/en/cervical-cancer-screening-programme
20. Finnish Cancer Registry. Cervical cancer screening; 2019.
21. Australian Government Department of Health. National Cervical Screening Program; 2017.
22. Cancer Registry of Norway. The Norwegian Cervical Cancer Screening Programme (NCCSP) [Internet]; 2019 [cited 2019 Jun 9]. Available from: https://www.kreftregisteret.no/en/screening/Cervical-Cancer-Screening-Programme/

23. NORDSCREEN. Cancer screening fact sheet [Internet]; 2017 [cited 2019 Jun 9]. Available from: https://nordscreen.org/wp-content/uploads/2017/05/Cervix-Fact-Sheet-Sweden-2017.pdf
24. Boom K, Lopez M, Daheri M, et al. Perspectives on cervical cancer screening and prevention: challenges faced by providers and patients along the Texas–mexico border. Perspect Public Health. 2018;139:199–205.
25. Watson M, Benard V, King J, et al. National assessment of HPV and Pap tests: changes in cervical cancer screening, National Health Interview Survey. Prev Med. 2017;100:243–247.
26. Saslow D, Solomon D, Lawson HW, et al. American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer. J Low Genit Tract Dis. 2012;16:175.
27. U.S. Food & Drug Administration. Premarket approval (PMA): aptima HPV assay [Internet]; 2011 [cited 2019 Apr 16]. Available from: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpma/pma.cfm?id=P100042.
28. U.S. Food & Drug Administration. New approaches in the evaluation for high-risk human papillomavirus nucleic acid detection devices [Internet]; 2019 [cited 2019 Apr 16]. Available from: https://www.fda.gov/media/122799/download
• **Describes FDA- approved HPV tests along with their indications for use**
29. Pantanowitz L, Hornish M, Goulart RA. The impact of digital imaging in the field of cytopathology. Cytojournal. 2009;6:6.
30. Campos NG, Tsu V, Jeronimo J, et al. Estimating the value of point-of-care HPV testing in three low- and middle-income countries: a modeling study. BMC Cancer. 2017;17:791.
31. Campos NG, Castle PE, Wright TC, et al. Cervical cancer screening in low-resource settings: a cost-effectiveness framework for valuing tradeoffs between test performance and program coverage. Int J Cancer. 2015;137:2208–2219.
32. Hu L, Bell D, Antani S, et al. An observational study of deep learning and automated evaluation of cervical images for cancer screening. J Natl Cancer Inst. 2019.
•• **Promising work toward incorporating machine learning into mobile colposcopy platform.**
33. Catarino R, Petignat P, Dongui G, et al. Cervical cancer screening in developing countries at a crossroad: emerging technologies and policy choices. World J Clin Oncol. 2015;6:281–290.
•• **Discusses challenges associated with implementing cervical cancer prevention programs in low-resource settings.**
34. Raifu AO, El-Zein M, Sangwa-Lugoma G, et al. Determinants of cervical cancer screening accuracy for visual inspection with acetic acid (VIA) and Lugol's Iodine (VILI) performed by nurse and physician. PLoS One. 2017;12: e0170631.
35. Silkensen SL, Schiffman M, Sahasrabuddhe V, et al. Is it time to move beyond visual inspection with acetic acid for cervical cancer screening? Glob Heal Sci Pract. 2018;6:242–246.
36. Gupta S, Palmer C, Bik EM, et al. Self-sampling for human papillomavirus testing: increased cervical cancer screening participation and incorporation in international screening programs. Front Public Health. 2018;6: 77.
37. Nelson EJ, Maynard BR, Loux T, et al. The acceptability of self-sampled screening for HPV DNA: a systematic review and meta-analysis. Sex Transm Infect. 2017;93:56–61.
• **Systematic review of the acceptability of HPV self-sampling among a large number of patients.**
38. Othman NH, Zaki FHM. Self-Collection Tools for Routine Cervical Cancer Screening: A Review. Asian Pac J Cancer Prev. 2014;15:8563–8569.
39. Madzima TR, Vahabi M, Lofters A. Emerging role of HPV self-sampling in cervical cancer screening for hard-to-reach women: focused literature review. Can Fam Physician. 2017;63:597–601.
40. Sankaranarayanan R. "See-and-treat" works for cervical cancer prevention: what about controlling the high burden in India? Indian J Med Res. 2012;135:576–579.
41. Gargano J, Meites E, Watson M, et al. Surveillance manual, chapter 5: human papillomavirus. Atlanta, Georgia: Centers for Disease Control and Prevention; 2009.
42. Wright TC, Stoler MH, Behrens CM, et al. Primary cervical cancer screening with human papillomavirus: end of study results from the ATHENA study using HPV as the first-line screening test. Gynecol Oncol. 2015;136:189–197.
43. Walboomers JMM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol. 1999;189:12–19.
44. Ying H, Jing F, Fanghui Z, et al. High-risk HPV nucleic acid detection kit–the careHPV test –a new detection method for screening. Sci Rep. 2015;4:4704.
45. Castle PE, Rodríguez AC, Burk RD, et al. Long-term persistence of prevalently detected human papillomavirus infections in the absence of detectable cervical precancer and cancer. J Infect Dis. 2011;203:814–822.
46. Yim E-K, Park J-S. The role of HPV E6 and E7 oncoproteins in HPV-associated cervical carcinogenesis. Cancer Res Treat. 2005;37:319.
47. Iftner T, Becker S, Neis K-J, et al. Head-to-head comparison of the RNA-based Aptima human papillomavirus (HPV) assay and the DNA-based Hybrid Capture 2 HPV test in a routine screening population of women aged 30 to 60 years in Germany. J Clin Microbiol. 2015;53:2509–2516.
48. Kelly H, Mayaud P, Segondy M, et al. A systematic review and meta-analysis of studies evaluating the performance of point-of-care tests for human papillomavirus screening. Sex Transm Infect. 2017;93:S36–S45.
49. Schweizer J, Lu PS, Mahoney CW, et al. Feasibility study of a human papillomavirus E6 oncoprotein test for diagnosis of cervical precancer and cancer. J Clin Microbiol. 2010;48:4646–4648.
50. Huh WK, Guido R. Transitioning from HPV 101 to HPV 202. Am J Obstet Gynecol. 2017;216:206–207.
51. Clifford GM, Smith JS, Plummer M, et al. Human papillomavirus types in invasive cervical cancer worldwide: a meta-analysis. Br J Cancer. 2003;88:63–73.
52. The FUTURE II Study Group. Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions. N Engl J Med. 2007;356:1915–1927.
53. Harper DM, Franco EL, Wheeler C, et al. Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial. Lancet. 2004;364:1757–1765.
54. Saslow D, Castle PE, Cox JT, et al. American Cancer Society Guideline for human papillomavirus (HPV) vaccine use to prevent cervical cancer and its precursors. CA Cancer J Clin. 2007; 57:7–28.
55. Oliver SE, Unger ER, Lewis R, et al. Prevalence of human papillomavirus among females after vaccine introduction—national Health and Nutrition Examination Survey, United States, 2003–2014. J Infect Dis. 2017;216:594–603.
56. Sahasrabuddhe VV, Luhn P, Wentzensen N. Human papillomavirus and cervical cancer: biomarkers for improved prevention efforts. Future Microbiol. 2011;6:1083–1098.
•• **Thoroughly describes established and promising biomarkers for cervical precancer detection.**
57. Dehn D, Torkko KC, Shroyer KR. Human papillomavirus testing and molecular markers of cervical dysplasia and carcinoma. Cancer Cytopathol. 2007;111: 1–14.
58. Wentzensen N, Clarke MA, Bremer R, et al. Clinical evaluation of human papillomavirus screening with p16/Ki-67 dual stain triage in a large organized cervical cancer screening program. JAMA Intern Med. 2019;179:881–888.
59. Peeters E, Wentzensen N, Bergeron C, et al. Meta-analysis of the accuracy of p16 or p16/Ki-67 immunocytochemistry versus HPV testing for the detection of CIN2+/CIN3+ in triage of women

with minor abnormal cytology. Cancer Cytopathol. 2019;127:169–180.
60. De Strooper LMA, Meijer CJLM, Berkhof J, et al. Methylation Analysis of the FAM19A4 Gene in Cervical Scrapes Is Highly Efficient in Detecting Cervical Carcinomas and Advanced CIN2/3 Lesions. Cancer Prev Res. 2014;7:1251–1257.
61. Verhoef VMJ, Heideman DAM, van Kemenade FJ, et al. Methylation marker analysis and HPV16/18 genotyping in high-risk HPV positive self-sampled specimens to identify women with high grade CIN or cervical cancer. Gynecol Oncol. 2014;135:58–63.
62. Verhoef VMJ, Bosgraaf RP, van Kemenade FJ, et al. Triage by methylation-marker testing versus cytology in women who test HPV-positive on self-collected cervicovaginal specimens (PROHTECT-3): a randomised controlled non-inferiority trial. Lancet Oncol. 2014;15:315–322.
63. Schmitz M, Eichelkraut K, Schmidt D, et al. Performance of a DNA methylation marker panel using liquid-based cervical scrapes to detect cervical cancer and its precancerous stages. BMC Cancer. 2018;18:1197.
64. Luttmer R, De Strooper LMA, Berkhof J, et al. Comparing the performance of FAM19A4 methylation analysis, cytology and HPV16/18 genotyping for the detection of cervical (pre)cancer in high-risk HPV-positive women of a gynecologic outpatient population (COMETH study). Int J Cancer. 2016;138:992–1002.
65. Lorincz AT, Brentnall AR, Scibior-Bentkowska D, et al. Validation of a DNA methylation HPV triage classifier in a screening sample. Int J Cancer. 2016;138:2745–2751.
66. Cook DA, Krajden M, Brentnall AR, et al. Evaluation of a validated methylation triage signature for human papillomavirus positive women in the HPV FOCAL cervical cancer screening trial. Int J Cancer. 2019;144:2587–2595.
67. Wentzensen N, Sun C, Ghosh A, et al. Methylation of HPV18, HPV31, and HPV45 Genomes and Cervical Intraepithelial Neoplasia Grade 3. JNCI J Natl Cancer Inst. 2012;104:1738–1749.
68. Sankaranarayanan R, Nene BM, Shastri SS, et al. HPV screening for cervical cancer in rural India. N Engl J Med. 2009;360:1385–1394.
69. Franco EL. Self-sampling for cervical cancer screening: empowering women to lead a paradigm change in cancer control. Curr Oncol. 2018;25: e1.
70. PAHO. Summary of commercially available HPV tests; 2016..
•• **Summarizes HPV tests for use in low-resource settings**
71. Abreu ALP, Souza RP, Gimenes F, et al. A review of methods for detect human Papillomavirus infection. Virol J. 2012;9:262.
72. Burd EM. Human papillomavirus laboratory testing: the changing paradigm. Clin Microbiol Rev. 2016;29:291–319.
•• **Describes and discusses laboratory-based HPV testing methods and technologies.**
73. Ying H, Jing F, Fanghui Z, et al. High-risk HPV nucleic acid detection kit-the careHPV test - a new detection method for screening. Sci Rep. 2014;4:4704.
74. World Health Organization. WHO prequalification of in vitro diagnostics: public report on Xpert® HPV; 2017.
75. Cuschieri K, Geraets D, Cuzick J, et al. Performance of a cartridge-based assay for detection of clinically significant human papillomavirus (HPV) infection: lessons from VALGENT (validation of HPV genotyping tests). Loeffelholz MJ, editor. J Clin Microbiol. 2016;54:2337–2342.
76. Molden T, Kraus I, Skomedal H, et al. PreTectTM HPV-Proofer: real-time detection and typing of E6/E7 mRNA from carcinogenic human papillomaviruses. J Virol Methods. 2007;142:204–212.
77. Kraus I, Molden T, Holm R, et al. Presence of E6 and E7 mRNA from human papillomavirus types 16, 18, 31, 33, and 45 in the majority of cervical carcinomas. J Clin Microbiol. 2006;44:1310–1317.
78. 1st WHO international standard for human papillomavirus (HPV) type 16 DNA, NIBSC code: 06/202, version 4.0. Hertfordshire, United Kingdom: National Institute for Biological Standards and Control; 2013.
79. Shah S, Senapati S, Klacsmann F, et al. Current technologies and recent developments for screening of HPV-associated cervical and oropharyngeal cancers. Cancers (Basel). 2016;8:85.
80. Levi AW, Bernstein JI, Hui P, et al. A comparison of the Roche cobas HPV test with the Hybrid Capture 2 test for the detection of high-risk human papillomavirus genotypes. Arch Pathol Lab Med. 2016;140:153–157.
81. Tin-Oo C, Hlaing HNT, Nandar CS, et al. Why the cost of purchasing the careHPV test in Myanmar was many times greater than that reported in the international literature. J Glob Oncol. 2018.
82. Vodicka EL, Babigumira JB, Mann MR, et al. Costs of integrating cervical cancer screening at an HIV clinic in Kenya. Int J Gynecol Obstet. 2017;136:220–228.
83. Jeronimo J, Bansil P, Lim J, et al. A multicountry evaluation of care HPV testing, visual inspection with acetic acid, and Papanicolaou testing for the detection of cervical cancer. Int J Gynecol Cancer. 2014;24:576–585.
84. Kang L-N, Jeronimo J, Qiao Y-L, et al. Optimal positive cutoff points for careHPV testing of clinician- and self-collected specimens in primary cervical cancer screening: an a from rural China. J Clin Microbiol. 2014;52:1954–1961.
85. Gage JC, Ajenifuja KO, Wentzensen N, et al. Effectiveness of a simple rapid human papillomavirus DNA test in rural Nigeria. Int J Cancer. 2012;131:2903–2909.
86. Wang M, Hu S, Zhao S, et al. Accuracy of triage strategies for human papillomavirus DNA-positive women in low-resource settings: a cross-sectional study in China. Chin J Cancer Res. 2017;29:496–509.
87. Segondy M, Kelly H, Magooa MP, et al. Performance of careHPV for detecting high-grade cervical intraepithelial neoplasia among women living with HIV-1 in Burkina Faso and South Africa: HARP study. Br J Cancer. 2016;115:425–430.
88. Obiri-Yeboah D, Adu-Sarkodie Y, Djigma F, et al. Self-collected vaginal sampling for the detection of genital human papillomavirus (HPV) using careHPV among Ghanaian women. BMC Womens Health. 2017;17:86.
89. Castle PE, Solomon D, Wheeler CM, et al. Human papillomavirus genotype specificity of Hybrid Capture 2. J Clin Microbiol. 2008;46:2595–2604.
90. Hsiang E, Little KM, Haguma P, et al. Higher cost of implementing Xpert MTB/RIF in Ugandan peripheral settings: implications for cost-effectiveness. Int J Tuberc Lung Dis. 2016;20:1212–1218.
91. MSF Access Campaign. Putting HIV and HCV to the test: A product guide for point-of-care CD4 tests and laboratory-based and point-of-care HIV and HCV viral load tests. 3rd ed. Geneva, Switzerland: Médecins Sans Frontières; 2017.
92. Einstein MH, Smith KM, Davis TE, et al. Clinical evaluation of the cartridge-based GeneXpert human papillomavirus assay in women referred for colposcopy. J Clin Microbiol. 2014;52:2089–2095.
93. Cuzick J, Cuschieri K, Denton K, et al. Performance of the Xpert HPV assay in women attending for cervical screening. Papillomavirus Res. 2015;1:32–37.
94. Castle PE, Smith KM, Davis TE, et al. Reliability of the Xpert HPV assay to detect high-risk human papillomavirus DNA in a colposcopy referral population. Am J Clin Pathol. 2015;143:126–133.
95. Toliman P, Badman SG, Gabuzzi J, et al. Field evaluation of Xpert HPV point-of-care test for detection of human papillomavirus infection by use of self-collected vaginal and clinician-collected cervical specimens. Diekema DJ, editor. J Clin Microbiol. 2016;54:1734–1737.
96. Zhao Y, Chen F, Li Q, et al. Isothermal amplification of nucleic acids. Chem Rev. 2015;115:12491–12545.
97. Cuzick J, Cadman L, Mesher D, et al. Comparing the performance of six human papillomavirus tests in a screening population. Br J Cancer. 2013;108:908–913.
•• **Compares performance of *digene* HC2, careHPV, Xpert HPV, and Aptima HPV.**
98. Hologic. Hologic Global Access Initiative [Internet]; 2019 [cited2019 Mar 29]. Available from: https://www.hologic.com/globalaccessinitiative

99. Jeantet D, Schwarzmann F, Tromp J, et al. NucliSENS® EasyQ® HPV v1 test – testing for oncogenic activity of human papillomaviruses. J Clin Virol. 2009;45:S29–S37.
100. Oliveira A, Verdasca N, Pista Â. Use of the NucliSENS EasyQ HPV assay in the management of cervical intraepithelial neoplasia. J Med Virol. 2013;85:1235–1241.
101. Rongioletti M, Papa F, Vaccarella C, et al. Clinical performance of HPV Oncotect compared to NucliSENS EasyQ Assay and its potential role over Papanicolaou test in detecting preneoplastic lesions of the cervix. Am J Clin Pathol. 2014;142: A228–A228.
102. Halfon P, Benmoura D, Agostini A, et al. Relevance of HPV mRNA detection in a population of ASCUS plus women using the NucliSENS EasyQ® HPV assay. J Clin Virol. 2010;47:177–181.
103. Fontecha N, Nieto MC, Andía D, et al. RNA extraction method is crucial for human papillomavirus E6/E7 oncogenes detection. Virol J. 2017;14:50.
104. PreTect. PreTect HPV-Proofer [Internet]; 2019 [cited2019 Mar 27]. Available from: https://www.pretect.no/pretecthpvproofer
105. Ratnam S, Coutlee F, Fontaine D, et al. Aptima HPV E6/E7 mRNA test is as sensitive as Hybrid Capture 2 assay but more specific at detecting cervical precancer and cancer. J Clin Microbiol. 2011;49:557–564.
106. Zhao F-H, Jeronimo J, Qiao Y-L, et al. An evaluation of novel, lower-cost molecular screening tests for human papillomavirus in rural China. Cancer Prev Res. 2013;6:938–948.
107. Petrone J With studies underway in South America and Asia, QuantuMDx readies POC platform for launch [Internet]. GenomeWeb; 2019 [cited 2019 Apr 21]. Available from: https://www.genomeweb.com/molecular-diagnostics/studies-underway-south-america-and-asia-quantumdx-readies-poc-platform-launch#.XL01lpNKiu5
108. Burn J. Company profile: QuantuMDx Group Limited. Pharmacogenomics. 2013;14:1011–1015.
• **Outlines the path forward in commercialization and scale-up for QuantuMDx.**
109. Ortega C, Steadman A, Nelis S, et al. Low cost diagnostic for the identification and typing of human papillomavirus to support cervical cancer screening in low-resource settings; 2018.
110. Rodriguez NM, Wong WS, Liu L, et al. A fully integrated paperfluidic molecular diagnostic chip for the extraction, amplification, and detection of nucleic acids from clinical samples. Lab Chip. 2016;16:753–763.
• **Promising approach toward point-of-care HPV detection appropriate for low-resource settings.**
111. Lafleur LK, Bishop JD, Heiniger EK, et al. A rapid, instrument-free, sample-to-result nucleic acid amplification test. Lab Chip. 2016;16:3777–3787.
• **Promising approach toward a fully-integrated isothermal nucleic acid amplification test.**
112. Kaur N, Toley BJ. Paper-based nucleic acid amplification tests for point-of-care diagnostics. Analyst. 2018;143:2213–2234.
113. Crannell ZA, Rohrman B, Richards-Kortum R. Equipment-free incubation of recombinase polymerase amplification reactions using body heat. Ugaz VM, editor. PLoS One. 2014;9.
114. Schweizer J, Berard-Bergery M, Bisht A, et al. E6 based rapid diagnostic test for cervical pre-cancer and cancer.
115. Rezhake R, Hu S-Y, Zhao S, et al. Eight-type human papillomavirus E6/E7 oncoprotein detection as a novel and promising triage strategy for managing HPV-positive women. Int J Cancer. 2019;144:34–42.
116. Madiedo M, Contreras S, Villalobos O, et al. Mobile colposcopy in urban and underserved suburban areas in Baja California. Opt Biophotonics Low-Resour Settings II. 2016; 9699: 96990I.
117. Thay S, Goldstein A, Goldstein LS, et al. Prospective cohort study examining cervical cancer screening methods in HIV-positive and HIV-negative Cambodian Women: a comparison of human papilloma virus testing, visualization with acetic acid and digital colposcopy. BMJ Open. 2019;9: e026887.
118. Mueller JL, Asma E, Lam CT, et al. International image concordance study to compare a point-of-care tampon colposcope with a standard-of-care colposcope. J Low Genit Tract Dis. 2017;21:112–119.
119. Asiedu MN, Agudogo J, Krieger MS, et al. Design and preliminary analysis of a vaginal inserter for speculum-free cervical cancer screening. PLoS One. 2017;12: e0177782.
120. Lam CT, Krieger MS, Gallagher JE, et al. Design of a novel low cost point of care tampon (POCkeT) colposcope for use in resource limited settings. PLoS One. 2015;10: e0135869.
• **Promising low-cost approach to mobile colposcopy.**
121. Lam CT, Mueller J, Asma B, et al. An integrated strategy for improving contrast, durability, and portability of a Pocket Colposcope for cervical cancer screening and diagnosis. PLoS One. 2018;13: e0192530.
122. Nessa A, Wistrand C, Begum SA, et al. Evaluation of stationary colposcope and the Gynocular, by the Swede score systematic colposcopic system in VIA positive women: a crossover randomized trial. Int J Gynecol Cancer. 2014;24:339–345.
• **Description and clinical evaluation of the Gynocular, a lower cost colposcopy technology.**
123. Kallner HK, Persson M, Thuresson M, et al. Diagnostic colposcopic accuracy by the Gynocular and a stationary colposcope. Int J Technol Assess Health Care. 2015;31:181–187.
124. Taghavi K, Banerjee D, Mandal R, et al. Colposcopy telemedicine: live versus static Swede score and accuracy in detecting CIN2+, a cross-sectional pilot study. BMC Womens Health. 2018;18:89.
125. Quinn MK, Bubi TC, Pierce MC, et al. High-resolution microendoscopy for the detection of cervical Nnoplasia in low-resource settings. PLoS One. 2012;7: e44924.
126. Grant BD, Fregnani JHTG, Possati Resende JC, et al. High-resolution microendoscopy: a point-of-care diagnostic for cervical dysplasia in low resource settings. Eur J Cancer Prev. 2017;26:63–70.
127. Hunt B, Fregnani JHTG, Schwarz RA, et al. Diagnosing cervical neoplasia in rural Brazil using a mobile van equipped with in vivo microscopy: a cluster-randomized community trial. Cancer Prev Res. 2018;11:359–370.
• **Clinical evaluation of the high-resolution microendoscope, an in vivo microscopy technology.**
128. Quang T, Schwarz RA, Dawsey SM, et al. A tablet-interfaced high-resolution microendoscope with automated image interpretation for real-time evaluation of esophageal squamous cell neoplasia. Gastrointest Endosc. 2016;84:834–841.
129. Grant BD, Quang T, Possati-Resende JC, et al. A mobile-phone based high-resolution microendoscope to image cervical precancer. PLoS One. 2019;14:e0211045.
130. Parra S, Keahey P, Schmeler K, et al. Development of a single-board computer high-resolution microendoscope (PiHRME) to increase access to cervical cancer screening in underserved areas. Adv Opt Biotechnol Med Surg XV. 2017.
131. Tang Y, Carns J. Line-scanning confocal microendoscope for nuclear morphometry imaging. J Biomed Opt. 2017;22:1.
132. Keahey P, Ramalingam P, Schmeler K, et al. Differential structured illumination microendoscopy for in vivo imaging of molecular contrast agents. Proc Natl Acad Sci. 2016;113:10769–10773.
133. Degueldre M, Vandromme J, de Wind A, et al. Real-time in-vivo microscopic imaging of the cervix using confocal laser endomicroscopy: preliminary observations and feasibility study. Eur J Cancer Prev. 2016;25:335–343.
134. Tan J, Quinn M, Pyman J, et al. Detection of cervical intraepithelial neoplasia in vivo using confocal endomicroscopy. BJOG. 2009;116:1663–1670.
135. Schlosser C, Bodenschatz N, Lam S, et al. Fluorescence confocal endomicroscopy of the cervix: pilot study on the potential and limitations for clinical implementation. J Biomed Opt. 2016;21: 126011.
136. Gallwas JKS, Turk L, Stepp H, et al. Optical coherence tomography for the diagnosis of cervical intraepithelial neoplasia. Lasers Surg Med. 2011;43:206–212.

137. Belinson SE, Ledford K, Rasool N, et al. Cervical epithelial brightness by optical coherence tomography can determine histological grades of cervical neoplasia. J Low Genit Tract Dis. 2013;17:160–166.
138. Zeng X, Zhang X, Li C, et al. Ultrahigh-resolution optical coherence microscopy accurately classifies precancerous and cancerous human cervix free of labeling. Theranostics. 2018;8:3099–3110.
139. Asiedu MN, Simhal A, Lam CT, et al. Image processing and machine learning techniques to automate diagnosis of Lugol's iodine cervigrams for a low-cost point-of-care digital colposcope. Conf Proc SPIE. 2018; 10485: 1048508.
140. Fernandes K, Cardoso JS, Fernandes J. Automated methods for the decision support of cervical cancer screening using digital colposcopies. IEEE Access. 2018;6:33910–33927.
141. Asgary R, Alcabes A, Feldman R, et al. Cervical cancer screening among homeless women of New York City shelters. Matern Child Health J. 2016;20:1143–1150.
142. Gatos KC. A literature review of cervical cancer screening in transgender men. Nurs Womens Health. 2018;22:52–62.