Utility of Xpert® HPV for cervical cancer screening of HIV-positive women

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Background

HIV-positive women are at high risk for HPV-related disease as a result of both behavioural and biological factors. More advanced HIV infection is associated with increased risk, while combination antiretroviral therapy (cART) reduces but does not eliminate the excess risk compared to HIV-negative women. We sought to evaluate the clinical utility of screening HIV-positive women using Cepheid GeneXpert® (Xpert® HPV), a real-time cartridge-based nucleic acid amplification test (NAAT) assay which enables partial genotyping based on detecting 14 high-risk HPV types, grouped into five channels, covering types 16, 18/45, 31/33/35/52/58, 51/59 and 39/56/66/68, respectively.

Methods and study population

Women aged 30–60 were recruited from the general population of Khayelitsha in the Western Cape, South Africa, with the aim of enrolling a similar number of HIV-positive and HIV-negative women. The women were taught to self-collect vaginal swabs which were screened using Xpert® HPV alongside clinician-collected cervical swabs. All women underwent a colposcopy at least once with histological sampling to detect the endpoint, which was cervical intraepithelial neoplasia grade 2, 3 or cancer (CIN2+) as determined by expert pathology review.

Table 1. Characteristics of study population

	HIV-negative (*n*=279)	HIV-positive* (*n*=250)
Age in years, mean (SD)	44.1 (9.4)	41.1 (7.3)
Parity, mean (range)	2.8 (0–12)	2.2 (0–7)
Education to grade 12 or above	31%	21%
In full-time employment	32%	29%
With cell phone	92%	92%
With internet access	38%	37%

* More than 80% of HIV-positive women were receiving cART.

Results

The study population comprised 250 HIV-positive and 279 HIV-negative women with characteristics as shown in Table 1. The prevalence of screen positivity for any of the five channels in clinician-collected cervical samples was 49.2% for HIV-positive and 16.1% for HIV-negative women. Self-collected vaginal samples showed a higher prevalence of 60.9% for HIV-positive and 25.9% for HIV-negative women. In each case HPV types 31/33/35/52/58 represented the most commonly detected channel (see Figure 1). The distribution of types appeared similar between HIV-positive and HIV-negative women, except for a slight excess of HPV 18/45 in HIV-positive women.

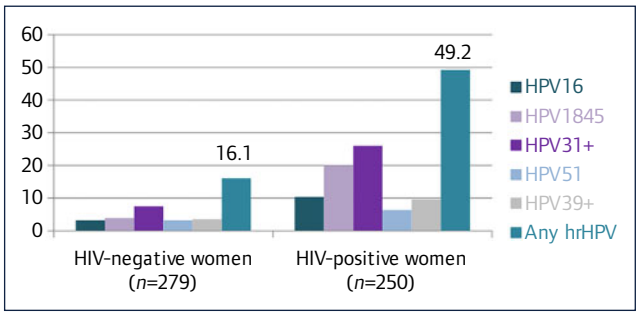

Figure 1. Prevalence of high-risk HPV in clinician-collected cervical samples using real-time PCR for 14 targeted HPV types in five channels: HPV 16; HPV 18/45; HPV 31/33/35/52/58; HPV 51/59; HPV 39/56/66/68

Table 2. Screening performance characteristics of Xpert® HPV in the study population

	Screen positive	Sensitivity	Specificity	Positive predictive value
HPV channels 16, 18/45, 31/33/35/52/58, 51/59, 39/56/66/68:				
Clinician-collected samples:				
HIV-negative women	16.1%	88.0%	89.2%	Not available
HIV-positive women	49.2%	95.0%	66.4%	Not available
Self-collected samples:				
HIV-negative women	25.9%	92.0%	77.4%	Not available
HIV-positive women	60.9%	95.8%	51.7%	Not available
HPV channels 16, 18/45, 31/33/35/52/58:				
Clinician-collected samples:				
HIV-negative women	9.7%	88.0%	93.0%	29.4%
HIV-positive women	41.6%	92.6%	71.6%	34.8%
Self-collected samples:				
HIV-negative women	Not available	92.0%	84.5%	Not available
HIV-positive women	Not available	91.6%	57.8%	Not available

Sensitivity of Xpert® HPV in predicting pathology-confirmed CIN2+ was high for both self- and clinician-collected samples in HIV-positive and HIV-negative women (Table 2). However, specificity was lower for HIV-positive compared with HIV-negative women and in self- rather than clinician-collected samples. Changing the definition of screen positivity to include samples testing positive on any of the first three channels, i.e. HPV types 16, 18/45 or 31/33/35/52/58, rather than all five led to a very slight decrease in sensitivity but improved specificity to over 90% for clinician-collected samples in HIV-negative women and over 70% in HIV-positive women.

Conclusion

HPV prevalence was high in HIV-positive women in our study population (49.2%). Xpert® HPV screening is highly sensitive in predicting pathology-confirmed CIN2+ but has limited specificity in HIV-positive women. Restricting the definition of screen positivity to the first three channels covering HPV types 16, 18/45 and 31/33/35/52/58 improved specificity in HIV-positive women, with minimal loss of sensitivity. This is of potential clinical utility in improving positive predictive value and reducing the number of women requiring further investigation.

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Practical considerations around using Xpert® HPV in low-income countries, focusing on Malawi

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Background

The worldwide burden of HPV-associated disease is concentrated in resource-constrained countries. Around 85% of all cervical cancers occur in these areas (see Figure 1) and it is the most common form of cancer in sub-Saharan Africa, South Asia and parts of Latin America. Additionally, almost nine out of 10 cervical cancer deaths occur in resource-constrained countries [1]. Devastatingly, it affects women in their prime child-bearing and productive years, and disproportionately affects the poorest and most vulnerable. As world population and life expectancy grow, the International Agency for Cancer Research predicts a 40% increase in cervical cancer by 2020.

Interventions based on cervical screening and HPV vaccination of girls can hugely influence country-level outcomes. Visual inspection with acetic acid (VIA) is the cornerstone of current screening as it is cheap, non-invasive and can be executed in low-tech health facilities with instant results. It is recommended by WHO [2] and has been adopted by 26 countries as their national screening strategy [3]. However, VIA is subjective, with high variability between evaluators even in quality settings. Low-grade lesions show as aceto-white, leading to overtreatment. HPV testing is more effective than VIA in reducing the prevalence of cervical intraepithelial neoplasia grades 2, 3 or cancer (CIN2+) [4].

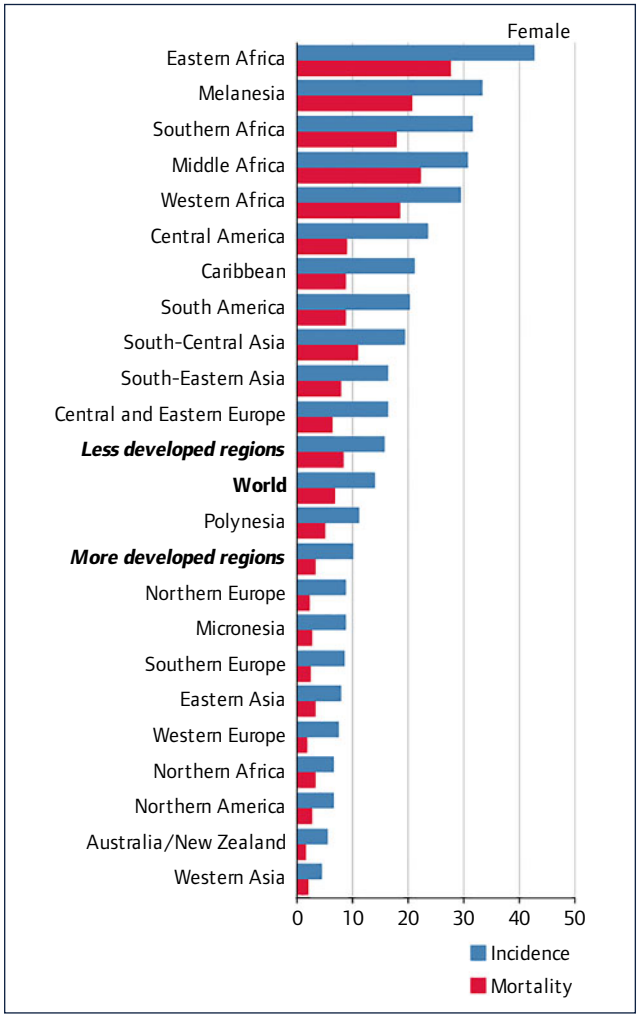

Figure 1. Age-standardised cervical cancer incidence and mortality rates by world area. Reproduced from [1]

The highest cervical cancer prevalence in the world is in Malawi, where it is the commonest cancer in women, accounting for 45% of cancers. The Ministry of Health estimates that if nothing is done, cases will increase by 60% by 2025. Malawi has a policy for VIA but this is difficult to deliver. We sought to deliver a same day 'screen and treat' programme using VIA and thermo-coagulation for treatment of early lesions [5].

Choice of HPV test is a difficult issue for low- and middle-income countries (LMIC) such as Malawi. A 2016 study [6] concluded that the 'HPV test global market is one of the most confusing, least regulated and with the most divergent products on the market'. The researchers found at least 193 distinct tests available (a 54.4% increase since 2012) with a further 127 test variants (78.8%). While this is probably an underestimate, most tests are oriented towards Western markets and despite reducing costs they are still too expensive for LMIC. Yet cost is by no means the only challenge in delivering HPV testing in LMIC. Many LMIC laboratories are unsuited to nucleic acid-based tests, having staff trained for microscopy, blood analytes and point-of-care testing for HIV and malaria, but not molecular testing. Equipment for high-throughput assays is usually prohibitively expensive, and takes up too much space. Individual country agents are lacking which inhibits access to technical support, maintenance and regular delivery of supplies. Disposal of waste fluids and plastics is a major problem. IT systems are limited and internet connectivity may be intermittent. Transportation of samples to testing laboratories may be difficult.

More specific problems are that there are many different collection devices and media for HPV testing. Media are often proprietary, associated with one test, and relatively expensive. Many are designed for cytology with high alcohol content and relatively large volume. They may require controlled temperature during transport and allow a limited storage time before testing. Testing procedures can be complex with a number of manual manipulations, giving potential for error or cross contamination. Turnaround times of over 2 hours render many HPV tests unsuitable for point-of-care use. Internal quality control and external quality assurance are additional issues, especially for small runs where the proportionate cost of controls can be high. Information on failure rates is essential.

Our aim was to identify means of reducing costs and waste from HPV testing while maintaining a reproducible assay that was simple to perform with a short turnaround time to allow a same-day

Table 1. Strengths and weaknesses of Xpert® HPV clinical testing in Africa

Strengths:

- Ease of use enabling several staff to carry out tests
- Partial genotyping, valuable for epidemiology
- 58-minute test turnaround allowing 2-hour clinic turnaround in a hospital setting
- Reproducible quality control with positive and negative internal controls introduced after every 20 specimens
- Potential for cheaper alternative collection media

Weaknesses:

- Procurement and transport
- Use of plastics
- Disposal of cartridges, media and large collection pots
- Appropriate temperature of clinic, transport to lab, and storage
- Cost – all HPV tests are still too expensive for routine use

Table 2. Prevalence of high-risk HPV in routinely screened women, using Xpert® HPV in accordance with manufacturer's instructions in VIA clinics in Nkhoma Hospital, Central Malawi [7]

Year of collection	2014	2015 to June	Total (%)	% of positives
Number tested	615	148	763	
With valid results	604	146	750 (98.3)	
HPV negative	484	117	601 (80.1)	
HPV positive	120	29	149 (19.9)	
HPV types:				
HPV 16	30	6	36 (4.8)	24.2
HPV 18/45	27	9	36 (4.8)	24.2
HPV 'other'	77	19	96 (12.8)	64.4
HPV 31/33/35/52/58	47	14	61 (8.1)	40.1
HPV 51/59	15	2	17 (2.3)	11.4
HPV 39/56/66/68	21	6	27 (3.6)	18.9
Multiple infections	17	7	24 (3.2)	16.1

Table 3. Results of paired Xpert® HPV testing with different media

PreservCyt® (PC):	HPV+	HPV-	Agreement	Kappa [95% CI]
Saline (*n*=52)			96.2%	88.5% [72.9–100]
HPV+	10	0		
HPV-	2	40		
Natrol® (*n*=93)			96.8%	88.4% [75.5–100]
HPV+	14	2		
HPV-	1	76		
NOVAprep HQ+ Orange® (*n*=96)			96.9%	92.2% [83.5–100]
HPV+	25	1		
HPV-	2	68		

'screen and treat' strategy, using testing as triage to VIA and treatment. We chose Xpert® HPV in the light of its strengths and weaknesses (see Table 1) [7].

Methods

We initially measured prevalence of high-risk HPV in routinely screened women using Xpert® HPV in accordance with the manufacturer's instructions, i.e. with PreservCyt® (PC) 20 mL in ThinPrep® pots. We then compared results with those obtained using alternative media and collection volumes and devices. We initially tried infusion saline because of delayed delivery of PC, but also investigated Natrol® and NOVAprep HQ+ Orange®, and self-collection of vaginal samples using cotton swabs, Quintips® and Vibabrushes®.

Results

Prevalence of HPV was 19.9% as shown in Table 2, with HPV 31/33/35/52/58 accounting for 40.1% of positive results. The age distribution of HIV and HPV positivity was as expected, with peak HPV incidence between 20 and 39, and peak HIV between 20 and 49. In terms of VIA outcomes, treatable lesions were predominantly seen in the 20–39 age group, with cancers and suspicious cancers at least a decade later.

Results of comparison testing using different collection media were as shown in Table 3 with saline, Natrol® and NOVAprep HQ+ Orange® all showing quite high Kappa values for agreement with PC despite relatively small numbers. Self-collection of vaginal samples was highly acceptable to women, and gave similar levels of HPV positivity when collected into PC with cotton swabs, Quintips® or Vibabrushes®. As with any HPV test, positivity rates were slightly higher for self-collected than clinician collected samples. We found 5 mL of PC was adequate for reproducible results. Plain cotton swabs (*n*=125) were by far the cheapest and gave remarkably comparable results provided that they were collected into PC and not sent dry to the laboratory. Quintips® (*n*=216) were easy to use with the manufacturer's or standard blood collection tubes and 4–5 mL PC added at the laboratory. Vibabrushes® (*n*=133) gave more invalid results in this small study, suggesting women found these harder to use. Further data were presented on Poster 143.

Discussion

Collection of 12 mL (NOVAprep HQ+ Orange®) or 20 mL (ThinPrep®) specimens in media designed for cytology is wasteful in LMIC. Collection systems including pots suited for cytology with cervix brushes are unnecessary, expensive and contain more plastic than desirable for disposal. Although sterile saline gave acceptable results in this small study, because of the potential for contamination it is unsuitable where transportation of samples is required. NOVAprep HQ+ Orange® gave the best HPV test result agreement with PC, and has a longer storage life (3 months rather than 3 weeks for clinical specimens) because it contains ethanol rather than methanol. However, a simpler medium would be preferable and we found only 5 mL of PC was needed for comparable results with provider-collected samples. Self-collection

of vaginal samples was acceptable to women in Nkhoma and plain cotton swabs gave reasonable detection of HPV if collected directly into medium in the clinic. Quintips® provided a simple, consistent system when used with manufacturer's or standard blood collection tubes, which are always available. This is easily transportable and it is straightforward for the laboratory to add 4–5 mL medium after arrival.

In conclusion, Xpert® HPV is straightforward to use with rapid turnaround and could potentially be offered as a near-patient test by clinical rather than laboratory staff. It should now be validated with larger numbers using self-taken vaginal samples and low-cost collection systems in LMIC. We owe it to girls, and their mothers and grandmothers, in Malawi and many other countries to advance not just the introduction of vaccine but also relevant HPV testing in 'screen and treat' programmes. My personal recommendations are that:

- All manufacturers, not just Cepheid, should agree universal collection systems, suitably priced for LMIC and not tied to a single test.
- Manufacturers should validate tests against a wider range of collection media, including simple, inexpensive media for use in LMIC.
- HPV tests should be as technically straightforward as possible for small laboratories or clinic staff to perform with minimal staff training.
- Turnaround time should be compatible with a single day visit and thus with 'see and treat' programmes.
- Discounted prices for LMIC are essential to achieve widespread implementation as prioritised by the Sustainable Development Goals.

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HPV diagnostic testing under the new Australian National Cervical Screening Program, including self-collection

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Background to the program

The Victorian Cytology Service (VCS) is a not-for-profit organisation that conducted around one-third-of-a-million cervical screening tests during 2016. Australia plans to introduce a renewed National Cervical Screening Program (NCSP) from 1 December 2017 (deferred from 1 May), using HPV-based primary screening with partial genotyping followed by reflex liquid-based cytology for HPV positives. Test selection will be 'open platform', based on criteria rather than a tendering process. Screening frequency will move from 2 to 5 years, starting at age 25.

Test and program criteria

The National Pathology Accreditation Advisory Council (NPAAC) released draft criteria as *Requirements for Laboratories Reporting Tests for the National Cervical Screening Program* on 15 June 2017 (www.health.gov.au/internet/main/publishing.nsf/Content/npaac-cervical-screening). I am a member of the committee that drew these up, and we aimed for simplicity, specifying that equipment/assays:

- Must satisfy Meijer criteria [1] for sensitivity, specificity and reproducibility, i.e.:
  - Non-inferiority to a validated reference assay
  - Clinical sensitivity for high-grade squamous intraepithelial lesion (HSIL) of ≥90% of the clinical sensitivity of Hybrid Capture® 2 (HC2) or an equivalent test in women ≥25 years of age
  - Clinical specificity for HSIL of ≥98% of HC2 or an equivalent test in women ≥25 years of age
  - Intra-laboratory reproducibility and inter-laboratory agreement with a lower confidence bound of 87%, with ≥500 samples tested and at least 30% HPV positive.
- Must be validated for primary population-based screening.
- Assay must contain a control to monitor inhibition and/or assay failure, and a control for cellularity to detect inadequate or empty cervical samples – these may be the same, e.g. beta-globin.
- Self-collected specimens must be tested using a PCR test.

We specified HC2 or an equivalent test because there are very few working HC2 systems in Australia, which is a large developed country. In addition, following analysis of data from our Compass trial of 121,000 women comparing liquid-based cytology to HPV screening, we require a minimum of 2000 screening tests to be assessed while samples remain valid for retesting (both HPV and cytology) to monitor positivity rates for HPV. This is viewed as an important safeguard for the program in Australia because in 2009 a change in the buffer for our bowel cancer screening program led to a drop in the positivity rate, resulting in suspension of the entire program for 6 months. Thus far five assays have been identified as fitting the criteria, and in order of publication these are the Roche cobas® 4800 [2], Abbott RealTi*m*e [3], BD Onclarity™ [4], Seegene Anyplex™ II [5] and Cepheid GeneXpert® HPV* [6]. Other tests are expected also to meet the criteria for approval. In terms of genotyping, cobas® 4800 and RealTi*m*e detect HPV 16, 18 or other (which includes the other 12 oncogenic types), Onclarity™ groups the 14 oncogenic types into nine groups while Xpert® presents the 14 oncogenic types in five groups. Anyplex™ II presents the 14 oncogenic HPV types individually. Technical and laboratory considerations are as shown in Table 1.

Self-collection as an alternative pathway for under-screened women

In Victoria, almost 90% of women diagnosed with cervical cancer are under-screened [7], despite a comparatively small state population of 5 million and an excellent cervical screening program. Accordingly, the renewed NCSP will allow under-screened or never-screened women to self-collect for HPV nucleic acid testing. The requirements are not yet finalised but it is proposed that women must be more than 2 years overdue for cervical screening to qualify. Self-collection will take place in a health service setting after consultation with a health practitioner. Women will be invited to attend and offered a speculum examination and Pap screen, with self-collection offered to those who decline.

We used dry flocked swabs for self-collection, which were resuspended in 4 mL of PreservCyt® once the sample arrived at the testing laboratory. Marathon Health provides care to the local community including Aboriginal women in a rural area of New South Wales, and 57 Aboriginal women agreed to take their own samples for HPV testing using a dry flocked swab. Results to date for this small number are: 1 HPV16 positive (1.75%), 1 HPV18 positive (1.75%), 6 non-HPV16/18 positive (10.5%), 40 not detected (70.2%), and 9 invalid results (15.8%). This rate of invalid results is of concern, since other studies have shown that under-screened women are less likely to return for a follow-up test following an invalid result, so they remain under-screened (Posters 59 and 59A show further information). In view of interest in point-of-care testing, we re-tested these samples using Xpert®. Definitive results were concordant except for three of the invalid results which became not detected, one which became HPV16 positive leading to colposcopy, and one which became

Table 1. HPV assays for use with renewed NCSP: technical and laboratory considerations. (Data is comparative to a validated reference assay)

HPV assay	Technical			Laboratory
	Target	Clinical sensitivity	Clinical specificity	Capacity (8 h)
Roche cobas® 4800	DNA: L1	0.99	1.00	288
Abbott RealTi*m*e	DNA: L1	1.00	1.01	288
BD Onclarity™	DNA: E6/E7	0.99	0.99	90
Seegene Anyplex™ II	DNA: L1	1.00	0.99	288
Cepheid GeneXpert®	DNA: E6/E7	1.00	1.00	7–560

Table 2. Comparison of results of cobas® and Xpert® in testing self-collected samples in under-screened women

		cobas® HPV test				
		HPV16	**HPV18**	**Other HPV**	**Negative**	**Invalid**
Xpert® HPV test	HPV16	1	0	0	0	1
	HPV18/45	0	1	2	0	0
	Other HPV	0	0	5	0	1
	Negative	0	0	0	40	3
	Invalid	0	0	0	0	4

other-positive leading to an offer of a Pap test or repeat testing after a year. Two samples positive for non-HPV16/18 on cobas® 4800 were positive for the 18/45 channel on Xpert®, consistent with type 45 infection. Also, a sample positive for non-HPV16/18 on cobas® 4800 was positive for two channels (31/33/35/52/58 and 51/59) on Xpert®, suggesting co-infection with at least two types. The results of the comparison are summarised in Table 2.

An important point is that the four remaining invalid results were genuinely invalid due to lack of cellular material in the samples. If tested using an assay lacking a cellularity control, these women would have been given negative results and wrongly advised to come back for re-testing in 5 years.

Summary and conclusion

The renewed NCSP will be the first open-platform screening program in the world. In our laboratory, prior to 1 December 2017, we will have five of the six HPV testing technologies likely to be available for the program. We are considering a 'horses for courses' approach and while Xpert® HPV might not be the easiest solution for 1200 samples a day, preliminary data suggest it may offer apparent advantages for point-of-care screening or self-collection. The new NCSP acknowledges that a screening program only works if people use it, and seeks to address this by offering a self-collection-based alternative pathway. Self-collected samples appear to have a higher rate (around 14–18% depending on population) of oncogenic HPV positivity, and Xpert® HPV appears less likely to give invalid results for such samples than the cobas® 4800 HPV test.

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Point-of-care HPV testing for cervical screening in high-burden, low-income settings

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Background

Papua New Guinea (PNG) is a low–middle-income country and the largest nation in Oceania. Among many problems in women's health, cervical cancer is the most common cancer and the largest cause of cancer deaths. It is estimated that PNG has one of the highest global burdens of cervical cancer, with incidence 6.3 times that of Australia and New Zealand (age standardised rates 34.5 *vs* 5.5/100,000), and mortality 13.5 times greater (21.7 *vs* 1.6/100,000). Despite intensive effort over the past 15 years, a non-governmental organisation-led Pap test programme achieved very limited coverage. Moreover, the challenging geography and context of PNG, with limited road infrastructure, mobile phone networks and postal systems, make it difficult to re-trace women identified as having high-grade disease. Following a review of the programme in 2009, a ministerial task force recommended evaluation of alternative approaches such as 'screen and treat'.

Visual inspection of the cervix with acetic acid (VIA) has been recommended as a screening strategy and implemented within other national programmes. Unfortunately, evaluation of VIA has yielded disappointing results in PNG and other settings. This led us to consider testing for high-risk HPV infection and to evaluate the Xpert® HPV test for point-of-care (PoC) use in PNG and other high-burden, low-income settings. Specifically, we evaluated self-collected vaginal specimens in comparison with clinician-collected cervical specimens, and the respective roles of PoC HPV testing and VIA in cervical screening. The study population comprised 1005 women aged 30–59 years attending Well Woman Clinics in Goroka and Mt Hagen during 2014–15, and our results are now being published [1].

Summary of findings

There was excellent agreement between self-collected vaginal and clinician-collected cervical specimens on PoC Xpert® HPV for the detection of underlying high-grade squamous intraepithelial lesions or above (HSIL) found on liquid-based cytology. VIA alone showed reasonable specificity but its sensitivity was low in comparison with PoC Xpert® testing for any high-risk HPV (hrHPV). A combined algorithm based on PoC Xpert® HPV testing followed by VIA for women testing positive showed higher specificity but much lower sensitivity than HPV testing alone. Selecting for HPV types 16/18/45 did not improve algorithm performance compared with performance based on any hrHPV type.

Discussion

Self-collection of specimens for PoC hrHPV testing offers enormous potential for opportunity savings in our setting, where around 12–15% of women have an hrHPV infection. Women who test hrHPV negative would not need a pelvic examination and can be advised to return at a later date for repeat testing, enabling scarce clinical resources to be focused on women who test hrHPV positive. Although in this study testing was performed by a member of the research team, in other studies we have trained clinical staff to conduct such tests without problems, showing this approach is feasible in routine clinical settings.

We found that sequential VIA after hrHPV testing dramatically lowered screening sensitivity compared with hrHPV testing alone. A study in Cameroon gave similar results, with sensitivity of 100.0 (95% CI 79.6–100.0) for HPV testing alone and 33.3 (95% CI 15.2–56.3) for HPV testing followed by VIA [2]. Similar results have also been reported in India [3]. Together, these findings suggest that the most appropriate role of visual inspection in future HPV-based POC 'test and treat' algorithms may be to guide cervical ablation (e.g. by ensuring that cryotherapy or thermo-coagulation ablates the entire transformation zone), rather than being used to inform decisions about who should or should not receive treatment.

In conclusion, a 'screen-and-treat' algorithm based on PoC Xpert® HPV testing of self-collected vaginal specimens had high sensitivity, specificity and predictive value compared with VIA examination alone and with a combined screening algorithm comprising HPV testing followed by VIA. Large-scale evaluation is warranted in PNG and other high-burden, low-income settings, to confirm these findings and to establish the health system implementation requirements, acceptability and cost-effectiveness of this PoC Xpert® HPV 'test and treat' approach.

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Panel discussion: key themes

HPV types for screening

A questioner cautioned against concluding that the P4/P5 Xpert® channels (HPV 51/59 and 39/56/66/68) are poorly associated with HSIL+ and that testing these channels could be omitted, in view of varying genotype prevalence in different countries. David Hawkes responded that in Australia there is monitoring of genotype prevalence, but also stressed the varying levels of risk posed by different types. The Australian programme used the term oncogenic HPV to avoid the apparently contradictory situation of informing women and their GPs of high-risk HPV infection but only intermediate cancer risk. The first three Xpert® channels covered the eight most prevalent HPV types, while the P4/P5 channels covered only four oncogenic types since HPV 66 and 68 are classified by WHO as 'probable' rather than oncogenic. Philip Castle emphasised that the guiding principle for a screening programme should be to optimise cancer prevention, rather than genotype prevalence in the general population. Given resource constraints, there is a trade-off between detecting more genotypes and screening larger numbers of women. Different genotypes show a gradient, with 16 as an exception. Screening for genotypes which make only a very small contribution to the cancer burden could have downstream consequences in terms of resources for further investigation of a larger number of women testing screen-positive.

Information for women undergoing screening

In response to a question about instructions for women coming for screening about, for instance, reproductive health, sexual practice and implications of hrHPV test results, Heather Cubie said that in Malawi written material is not given out but a clear educational message is delivered by a team of experienced and expert providers, and women have the time to ask as many questions as they wish. A standard flip-chart is used to ensure consistent and understandable information.

Low medium volume

A question was raised as to whether 4–5 mL media volumes were sufficient to prevent sample degradation. A cellularity control might not detect this, given some evidence that a study showing that in urine HPV DNA decays more rapidly than human DNA. Heather Cubie responded that problems had not been seen and the sample invalidity rate was very low, especially when using Quintips®. David Hawkes mentioned a study at VCS which showed 92% agreement between practitioner-collected cervical flocked swabs left on a bench for a week before addition of preservative with practitioner-collected brush samples placed straight into preservative. Further studies were continuing including comparisons between self- and practitioner-collected flocked swabs on a range of hrHPV tests, in order to have reliable data before the start of the renewed national screening programme.

Visualisation with acetic acid following HPV testing

Lynette Denny suggested that visualisation following a positive PoC hrHPV result should be considered as a safety check rather than a diagnostic intervention which presents a barrier to 'screen and treat'. Criteria such as >75% of the cervix showing as aceto-white, evidence of cancer or vaginal wall collapse would indicate women unsuitable for immediate cryotherapy and requiring specialist referral. Andrew Vallely agreed that visualisation was important to ensure the entire transition zone is visible and that lesions can be treated correctly. Philip Castle argued for new terminology to differentiate screening/diagnostic VIA from the same procedure being used after a positive hrHPV test, and suggested visual assessment for treatment or VAT. This would be helpful in emphasising the very different purpose of the examination to wider non-specialist audiences.

Silvia Franceschi expressed concerns that while pre-treatment visualisation was clinically logical, its continued use would be a strong obstacle to 'screen and treat' programmes because of the need for trained and experienced staff. Louise Kuhn responded that while VIA-based screening programmes had encountered this problem, hrHPV testing followed by visual assessment for treatment offered the advantage of screening out the 80% or so women who test negative. Only women testing positive would need examination, requiring a considerably smaller staff resource. Heather Cubie emphasised the importance of not only initial but continued regular training and at least annual assessment of competence for staff performing visualisation.