Title: **Field evaluation of the Xpert® HPV Point of Care Test for the detection of human papillomavirus infection using self-collected vaginal and clinician-collected cervical specimens**

Running Title: **Field evaluation of Xpert® HPV Test at point-of-care**

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ABSTRACT

The World Health Organization has recommended that testing for high-risk human papillomavirus (hrHPV) infection be incorporated into cervical screening programs in all settings worldwide. In many high-burden, low-income countries it will not be feasible to achieve high cervical screening coverage using hrHPV assays that require clinician-collected samples. We conducted the first evaluation of self-collected vaginal specimens compared with clinician-collected cervical specimens for the detection of hrHPV infection using the Xpert® HPV Test. Women aged 30-54 years attending two well woman clinics in Papua New Guinea were invited to participate and provided self-collected vaginal and clinician-collected cervical cytobrush specimens. Both were tested at point-of-care using the Xpert® HPV Test. Women were given their cervical test result the same day. Those with a positive hrHPV test and a positive examination on visual inspection of the cervix with acetic acid were offered same-day cervical cryotherapy. A total of 1005 women were enrolled with 124 (12.3%; 95%CI: 10.3, 14.4) positive for any hrHPV infection. There was 99.4% overall percentage agreement (OPA) between vaginal and cervical tests for HPV-16 (95%CI: 98.9, 99.9); 98.5% OPA for HPV-18/45 (95%CI: 97.7, 99.3); 94.4% OPA for other hrHPV infections (95%CI: 92.9, 95.9); and 93.4% OPA for all hrHPV types combined (95%CI: 91.8, 95.0). Self-collected vaginal specimens had excellent agreement with clinician-collected cervical specimens for the detection of hrHPV infection using the Xpert® HPV Test. This approach provides for the first time an opportunity to incorporate point-of-care hrHPV testing into clinical cervical screening algorithms in high-burden, low-income settings.

Keywords: HPV; point of care; cervical screening; Papua New Guinea

INTRODUCTION

The recognition that infection with certain high-risk types of human papillomavirus (hrHPV) is the primary cause of both cervical pre-cancer and cancer led to the development of new technologies that would allow hrHPV DNA to be detected as part of population-based screening. These tests are more sensitive than cytology for the detection of high grade cervical intraepithelial neoplasia (CIN) and invasive disease, and have comparable specificity(1, 2); and their potential efficacy for population-based cervical screening has been conclusively demonstrated in large-scale randomised trials and prospective studies(3-5). These findings led to recommendations in Europe, the United States, Australia and other high-income settings for cervical screening programs to incorporate hrHPV DNA testing(2, 5-7). In this rapidly developing environment, and based on trials directly comparing HPV screening with cytology(3-5), the World Health Organization recently recommended that hrHPV testing be incorporated into cervical screening programs in low-and middle-income countries (LIMC), particularly where cytological testing is not available and where visual inspection of the cervix after the application of acetic acid or Lugol's iodine (VIA/VILI) are the principal cervical screening strategies(8).

The Xpert® HPV Test (GeneXpert; Cepheid, Sunnyvale, CA) is a newly available, rapid, fully-automated and easy to use non-batch test for hrHPV infection that is as accurate as laboratory-based nucleic acid amplification tests (NAAT)(2, 9). Xpert® HPV compared favourably with the FDA-approved Cobas 4800 (Roche Molecular Systems, Pleasanton, CA) and Hybrid Capture 2 (hc2; Qiagen, Germantown, MD) assays for the detection of hrHPV using clinician-collected cervical specimens(2, 9), and had comparable sensitivity, specificity and positive predictive value to these assays for high grade CIN(2). Disposable cartridges hold the reagents, primers and probes for the simultaneous detection of 14 hrHPV types responsible for over 95% of cervical cancers (HPV-16, -18,

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-31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66 and -68), a human reference gene, and an internal Probe Check Control (PCC)(2). The system monitors the presence of inhibitors in the real-time polymerase chain reaction (PCR) assay to signal a potentially false negative result. Test results are available in 60 minutes and are displayed on the accompanying laptop, typically as three outputs: 'HPV-16'; 'HPV 18/45'; and 'Other HR HPV' (a summary of test results for HPV-31, -33, -35, -39, -51, -52, -56, -58, -59, -66 and -68). The Xpert HPV Test uses the same Cepheid GeneXpert platform that has now been widely introduced for the diagnosis of tuberculosis in LMIC settings worldwide. The availability of a test for hrHPV DNA that uses this same platform represents an opportunity for the first time to integrate clinic-based hrHPV testing within same-day 'test and treat' cervical screening programs in LMIC countries(8), particularly if self-collected specimens were proven to be as accurate as clinician-collected specimens for the detection of hrHPV infection

We have previously evaluated the GeneXpert platform for point-of-care testing and treatment of *C. trachomatis, N. gonorrhoeae* and *T. vaginalis* in routine clinic settings in Australia(10), and are currently evaluating this approach among antenatal women in Papua New Guinea(11). In this paper we report findings from the first evaluation of self-collected vaginal specimens compared with clinician-collected cervical specimens for the detection of hrHPV infection using the Xpert® HPV Test conducted at point-of-care in the high-burden, low-income setting of Papua New Guinea(12-14).

MATERIALS AND METHODS

Setting

Papua New Guinea has among the highest estimated burdens of cervical cancer globally, with incidence 6.3 times that of Australia and New Zealand (age standardized rates 34.5 *vs.* 5.5/100,000),

and mortality 13.5 times greater (21.7 *vs.* 1.6/100,000)(12, 13). Cervical cancer is the most common cancer among women in PNG and results in an estimated 1,500 deaths per year(12-14).

The current study was carried out at two well woman clinics (one in Goroka, Eastern Highlands Province; one in Mt Hagen, Western Highlands Province). These clinics were established by provincial health authorities to provide routine Pap test based cytology screening, and in recent years have collaborated with our research group to evaluate alternative cervical screening strategies such as VIA(14, 15). Information about cervical screening services provided at these clinics is communicated to women living in local catchment communities through community and clinic-based health talks by health facility staff, and through local radio announcements and media releases. No study-specific community activities or media announcements were carried out prior to the start of the current study.

Study population and design

Women aged 30-59 years attending for routine cervical screening were provided with information about the study whilst waiting to be seen and were enrolled consecutively into the study. Following written informed consent, a female research nurse/health extension officer (HEO) conducted a short face-to-face interview in which socio-demographic, behavioural and clinical information were collected. Women were then instructed how to obtain a single self-collected approximately 'mid-cavity' vaginal cytobrush specimen. A pictorial guide, piloted in our earlier research in this setting, was used to explain how the procedure should be carried out, including the approximate location for specimen collection within the vagina, and any questions or concerns discussed. Self-collection was conducted in a dedicated private room in each study clinic. Participants then underwent a gynaecological examination in which a single clinician-collected cervical cytobrush specimen was collected immediately prior to VIA examination.

Cervical and vaginal cytobrush specimens were placed in ThinPrep PreservCyt® (Hologic, Marlborough, MA) immediately after collection. Xpert® HPV testing of cervical and vaginal specimens was conducted side-by-side on a clinic-based GeneXpert machine operated by a trained member of the clinical research team in accordance with manufacturer's instructions. Women were provided with their cervical Xpert® HPV test result the same day. Those with a positive cervical hrHPV test and a positive VIA examination were offered same-day ablative cervical cryotherapy.

Ethical considerations

Approval was provided by the Medical Research Advisory Committee of the PNG National Department of Health (MRAC#14.28), the Institutional Review Board of the PNG Institute of Medical Research (IRB#1306); and by the Human Research Ethics Committee of UNSW Australia (HREC UNSW #HC13268). Written informed consent (signature or witnessed thumbprint) was obtained from all participants prior to enrolment.

Statistical analysis

Test result data were automatically stored by the GeneXpert-associated laptop computer. Results were also written into a daily test results log, and entered into a study-specific MS Excel database at each clinic site by a trained member of the clinical research team. At the end of the study the MS Excel database was checked for completeness and all entries verified against the GeneXpert laptop and written test results logs.

Positive, negative and overall percentage agreement (PPA, NPA, OPA) between cervical and vaginal specimens were calculated using standard methods(16) for (a) HPV-16; (b) HPV-18/45; (c) Other

hrHPV infection (HPV-31, -33, -35, -39, -51, -52, -56, -58, -59, -66 and -68); and (d) for any hrHPV infection (i.e. any one or more of the 14 hrHPV types detected by the Xpert HPV Test). The kappa statistic was calculated with 95% confidence intervals (CI) for test scenarios (a) – (d) above using STATA 12.1 (StataCorp, College Station, TX). A kappa of 0.41 – 0.60 was considered to indicate moderate agreement; 0.61 – 0.80, substantial agreement; and 0.81 – 1.00, excellent agreement(17).

RESULTS

A total of 1005 women were enrolled in Goroka (n=614) and Mt Hagen (n=391) in the period October 2014 – October 2015. All women invited to participate subsequently enrolled, successfully collected a mid-cavity vaginal specimen, and completed study procedures. None of the women invited to participate refused to do so and there were no withdrawals post-enrolment.

Based on cervical Xpert® HPV Test results, the prevalence of HPV-16 was 3.5% (95%CI: 2.3, 4.7); HPV-18/45, 1.6% (95%CI: 0.8, 2.4); other hrHPV, 9.0% (95% CI: 7.2, 10.8); and 12.3% (95%CI: 10.2, 14.4) for all hrHPV types combined. There was 99.4% overall percentage agreement (OPA) between vaginal and cervical tests for HPV-16 (95%CI: 98.9, 99.9); 98.5% OPA for HPV-18/45 (95%CI: 97.7, 99.3); 94.4% OPA for other hrHPV infections (95%CI: 92.9, 95.9); and 93.4% OPA for all hrHPV types combined (95%CI: 91.8, 95.0) (Table 1). Mean cycle thresholds for concordant positive vaginal and cervical tests were similar (e.g. for HPV-16, the mean threshold for positive vaginal tests was 29.78, and for positive cervical tests was 30.86; data not shown).

There were six disagreements between vaginal and cervical Xpert HPV tests for HPV-16; nine disagreements for HPV 18/45; and 32 disagreements for other hrHPV types. Of all the disagreements,

39/47 (83.0%) were positive on the vaginal specimen and negative on the cervical specimen, and discrepant vaginal tests were positive at high cycle thresholds (Table 2).

DISCUSSION

Self-collected vaginal specimens compared favourably to clinician-collected cervical specimens for the detection of hrHPV infection using the Xpert® HPV Test among women attending routine cervical screening services in Papua New Guinea. The absence of refusals to participate and lack of study withdrawals suggests a high degree of acceptability of specimen self-collection and is consistent with our earlier research in this setting where self-collection was used(11). Previous studies have demonstrated that the performance of laboratory-based molecular assays for the detection of HPV infection using self-collected specimens are comparable to clinician-collected specimens(18), but none investigated approaches with the potential for application at point-of-care. The strategy evaluated in the current study provides for the first time an opportunity to incorporate point-of-care hrHPV testing into clinical cervical screening algorithms in high-burden, low-income settings. A caveat is that although the Xpert® HPV Test has excellent performance characteristics compared with FDA-approved hrHPV assays for the detection of hrHPV using cervical specimens(2, 9), and high overall percentage agreement was observed between self-collected vaginal and clinician-collected cervical specimens in the current study, before point-of-care self-collection could be recommended as part of cervical screening algorithms, the performance of Xpert® HPV *vs.* Cobas 4800 and hc2 using vaginal specimens needs to be conclusively demonstrated. It will also be important to evaluate vaginal self-collection for the detection of cervical disease biomarkers.

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An *a priori* assumption in the current study was that vaginal specimens would be less sensitive for the detection of hrHPV compared with cervical specimens. Comparison of mean cycle threshold data among concordant paired test results suggests this is not the case, whilst the unexpected finding of the high proportion (83.0%) of paired test disagreements in which the vaginal test was positive indicates that the vaginal test may actually have greater sensitivity. An alternative explanation is that cervical mucus, or cervical discharge due to concomitant *C. trachomatis*, *N gonorrhoeae* or other sexually transmitted infections, may have introduced PCR inhibitors that affected the performance of the Xpert HPV test, although this seems unlikely given the presence of internal controls that are integral to the GeneXpert platform. Testing stored paired specimens by FDA-approved HPV assays, for cervical biomarkers and for the presence of *C. trachomatis*, *N gonorrhoeae* and other STIs will help clarify these findings..

A field trial to evaluate point-of-care Xpert® HPV testing plus VIA examination compared with standard routine care (VIA alone) for the detection and treatment of cervical pre-cancer lesions is expected to start enrolment in Papua New Guinea in 2016. If self-collection is proven to have comparable performance characteristics to clinician-collected specimens for the detection of hrHPV infection, the former will be used as the primary collection method in this trial. The study will also evaluate the cost-effectiveness, health system implementation requirements, and acceptability of the combined screening algorithm, and its findings are expected to inform international guidelines on cervical screening in high-burden, low-income settings.

FUNDING INFORMATION

This work was funded through the following research grants: National Health and Medical Research Council of Australia (NHMRC Training Fellowship Grant 1013209 to A. Vallely; NHMRC Program

214 Grant 1071269 to J. Kaldor); Papua New Guinea Institute of Medical Research, Internal Competitive
215 Research Award Scheme (ICRAS Grant 297/1 to A. Vallely). We also acknowledge the generous core
216 funding support provided to the Papua New Guinea Institute of Medical Research by the Australian
217 Aid Program, Department of Foreign Affairs and Trade, Government of Australia.
218
219 None of the funders or other organizations listed above had any role in study design, data collection
220 and interpretation, or the decision to submit the work for publication.
221
222 **ACKNOWLEDGMENTS**
223 We thank the women who took part in this research, and acknowledge the support of their families
224 and communities, without which this work would not have been possible. We also thank Cepheid
225 (Sunnyvale, CA) for generously donating Xpert® HPV Test cartridges for this project.
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285 **TABLE 1. Comparison of Xpert® HPV Test results using paired vaginal and cervical specimens**

HPV-16		Cervical specimen				
		Positive	Negative	Total	PPA (95% CI)	94.3 (92.8, 95.8)
Vaginal specimen	Positive	33	4	37	NPA (95% CI)	99.6 (99.2, 100.0)
	Negative	2	966	968	OPA (95% CI)	99.4 (98.9, 99.9)
	Total	35	970	**1005**	**Kappa** (95% CI)	**0.91 (0.86, 0.97)**
HPV-18/45		Cervical specimen				
		Positive	Negative	Total	PPA (95% CI)	81.3 (78.8, 83.8)
Vaginal specimen	Positive	13	12	25	NPA (95% CI)	98.8 (98.1, 99.5)
	Negative	3	977	980	OPA (95% CI)	98.5 (97.7, 99.3)
	Total	16	989	**1005**	**Kappa** (95% CI)	**0.63 (0.48, 0.77)**
Other hrHPV		Cervical specimen				
		Positive	Negative	Total	PPA (95% CI)	91.1 (89.3, 92.9)
Vaginal specimen	Positive	82	48	130	NPA (95% CI)	94.8 (93.4, 96.2)
	Negative	8	867	875	OPA (95% CI)	94.4 (92.9, 95.9)
	Total	90	915	**1005**	**Kappa** (95% CI)	**0.72 (0.65, 0.79)**
All hrHPV		Cervical specimen				
		Positive	Negative	Total	PPA (95% CI)	90.3 (88.4, 92.2)
Vaginal specimen	Positive	112	54	166	NPA (95% CI)	93.9 (92.4, 95.4)
	Negative	12	827	839	OPA (95% CI)	93.4 (91.8, 95.0)
	Total	124	881	**1005**	**Kappa** (95% CI)	**0.74 (0.70, 0.79)**

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287 CI: confidence interval; PPA: Positive Percentage Agreement; NPA: Negative Percentage Agreement; OPA: Overall Percentage Agreement

288 **TABLE 2. Summary of disagreement between Xpert® HPV Test results for vaginal and cervical specimens**

	HPV-16	HPV-18/45	Other hrHPV	Total
No. disagreements between vaginal and cervical test results	6	9	32	47
No. disagreements for which vaginal test POS / cervical test NEG (%)	4 (66.7)	7 (77.8)	28 (87.5)	39 (83.0)
Mean cycle threshold where vaginal test POS / cervical test NEG	32.65	36.46	34.89	34.94

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