AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology

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

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The World Health Organization has recommended that testing for high-risk human papillomavirus (HPV) (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 to 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 specimen types were tested at the point of care by using the Xpert HPV test. Women were given their cervical test result the same day. Those with a positive hrHPV test and positive examination upon visual inspection of the cervix with acetic acid were offered same-day cervical cryotherapy. A total of 1,005 women were enrolled, with 124 (12.3%; 95% confidence interval [CI], 10.3%, 14.4%) being positive for any hrHPV infection. There was a 99.4% overall percent agreement (OPA) between vaginal and cervical tests for HPV-16 (95% CI, 98.9%, 99.9%), a 98.5% OPA for HPV-18/45 (95% CI, 97.7%, 99.3%), a 94.4% OPA for other hrHPV infections (95% CI, 92.9%, 95.9%), and a 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.

The recognition that infection with certain high-risk types of human papillomavirus (HPV) (hrHPV) is the primary cause of both cervical precancer 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 specificities (1, 2), and their potential efficacy for population-based cervical screening has been conclusively demonstrated in large-scale randomized 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 (LIMCs), particularly where cytological testing is not available and where visual inspection of the cervix after the application of acetic acid (VIA) or visual inspection after the application of Lugol's iodine (VILI) is the principal cervical screening strategy (8).

The Xpert HPV test (GeneXpert; Cepheid, Sunnyvale, CA) is a newly available, rapid, fully automated, and easy-to-use nonbatch test for hrHPV infection that is as accurate as laboratory-based nucleic acid amplification tests (NAATs) (2, 9). Xpert HPV compared favorably to 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 sensitivity, specificity, and positive predictive values comparable to those of the above-mentioned 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, -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 a real-time PCR assay to signal a

Received 9 March 2016 **Returned for modification** 28 March 2016
Accepted 31 March 2016

Accepted manuscript posted online 13 April 2016

Citation Toliman P, Badman SG, Gabuzzi J, Silim S, Forereme L, Kumbia A, Kombuk B, Kombati Z, Allan J, Munnull G, Ryan C, Vallely LM, Kelly-Hanku A, Wand H, Mola GDL, Guy R, Siba P, Kaldor JM, Tabrizi SN, Vallely AJ. 2016. 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. J Clin Microbiol 54:1734–1737. doi:10.1128/JCM.00529-16.

Editor: D. J. Diekema, University of Iowa College of Medicine

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potentially false-negative result. Test results are available in 60 min and are displayed on the accompanying laptop, typically as three outputs: "HPV-16," "HPV-18/45," and "other hrHPV" (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 into same-day "test-and-treat" cervical screening programs in LMICs (8), particularly if self-collected specimens were proven to be as accurate as clinician-collected specimens for the detection of hrHPV infection.

We previously evaluated the GeneXpert platform for point-of-care testing and treatment of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* in routine-clinic settings in Australia (10) and are currently evaluating this approach among antenatal women in Papua New Guinea (A. Vallely, presented at the World STI & HIV Congress 2015, Brisbane, Australia, 13 to 16 September 2015). 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 the 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 an incidence 6.3 times higher than that of Australia and New Zealand (age-standardized rates, 34.5 versus 5.5/100,000) and a mortality rate that is 13.5 times higher (21.7 versus 1.6/100,000) (12, 13). Cervical cancer is the most common cancer among women in Papua New Guinea and results in an estimated 1,500 deaths per year (12–14).

This study was carried out at two well-woman clinics (one in Goroka, Eastern Highlands Province, and 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; A. Vallely, presented at the 51st Annual Symposium of the Medical Society of PNG, Port Moresby, Papua New Guinea, 2015). 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 present study.

Study population and design. Women aged 30 to 59 years attending clinics for routine cervical screening were provided information about the study while 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 sociodemographic, behavioral, and clinical information was collected. Women were then instructed how to obtain a single, self-collected, approximately "midcavity" vaginal cytobrush specimen. A pictorial guide, piloted in our previous study 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 gynecological examination in which a single clinician-collected cervical cytobrush specimen was collected immediately prior to VIA examination.

Cervical and vaginal cytobrush specimens were placed into 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 the manufacturer's instructions. Women were given 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 (MRAC) of the Papua New Guinea National Department of Health (approval number 14.28), the Institutional Review Board (IRB) of the Papua New Guinea Institute of Medical Research (approval number 1306), and the Human Research Ethics Committee (HREC) of UNSW Australia (approval number HC13268). Written informed consent (signature or witnessed thumbprint) was obtained from all participants prior to enrollment.

Statistical analysis. Test result data were automatically stored by a GeneXpert-associated laptop computer. Results were also written into a daily test result 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 were verified against the GeneXpert laptop and written test result logs.

Positive percent agreement (PPA), negative percent agreement (NPA) and overall percent agreement (OPA) between cervical and vaginal specimens were calculated by using standard methods (16) for (i) HPV-16, (ii) HPV-18/45, (iii) other hrHPV infections (HPV-31, -33, -35, -39, -51, -52, -56, -58, -59, -66, and -68), and (iv) 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 (CIs) for the test scenarios described above by using STATA 12.1 (StataCorp, College Station, TX). A kappa value of 0.41 to 0.60 was considered to indicate moderate agreement, a kappa value of 0.61 to 0.80 indicated substantial agreement, and a kappa value of 0.81 to 1.00 indicated excellent agreement (17).

RESULTS

A total of 1,005 women were enrolled in Goroka ($n = 614$) and Mt Hagen ($n = 391$) in the period from October 2014 to October 2015. All women invited to participate subsequently enrolled, successfully collected a midcavity vaginal specimen, and completed study procedures. None of the women invited to participate refused to do so, and there were no withdrawals postenrollment.

Based on cervical Xpert HPV test results, the prevalence of HPV-16 was 3.5% (95% CI, 2.3%, 4.7%), that of HPV-18/45 was 1.6% (95% CI, 0.8%, 2.4%), that of other hrHPVs was 9.0% (95% CI, 7.2%, 10.8%), and that of all hrHPV types combined was 12.3% (95% CI, 10.2%, 14.4%). There was 99.4% 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 threshold values 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 that for positive cervical tests was 30.86 [data not shown]).

There were 6 disagreements between vaginal and cervical Xpert HPV tests for HPV-16, 9 disagreements for HPV-18/45, and 32 disagreements for other hrHPV types. Of all the disagreements, 39/47 (83.0%) results were positive for the vaginal specimen and negative for the cervical specimen, and discrepant vaginal test results were positive at high cycle threshold values (Table 2).

TABLE 1 Comparison of Xpert HPV test results using paired vaginal and cervical specimens

Virus and vaginal specimen type	No. of cervical specimens			PPA (%) (95% CI), NPA (%) (95% CI), OPA (%) (95% CI), kappa value (95% CI)
	Positive	Negative	Total	
HPV-16				94.3 (92.8, 95.8), 99.6 (99.2, 100.0), 99.4 (98.9, 99.9), 0.91 (0.86, 0.97)
Positive	33	4	37	
Negative	2	966	968	
Total	35	970	1,005	
HPV-18/45				81.3 (78.8, 83.8), 98.8 (98.1, 99.5), 98.5 (97.7, 99.3), 0.63 (0.48, 0.77)
Positive	13	12	25	
Negative	3	977	980	
Total	16	989	1,005	
Other hrHPVs				91.1 (89.3, 92.9), 94.8 (93.4, 96.2), 94.4 (92.9, 95.9), 0.72 (0.65, 0.79)
Positive	82	48	130	
Negative	8	867	875	
Total	90	915	1,005	
All hrHPVs				90.3 (88.4, 92.2), 93.9 (92.4, 95.4), 93.4 (91.8, 95.0), 0.74 (0.70, 0.79)
Positive	112	54	166	
Negative	12	827	839	
Total	124	881	1,005	

DISCUSSION

Self-collected vaginal specimens compared favorably to clinician-collected cervical specimens for the detection of hrHPV infection using the Xpert HPV test among women attending clinics for routine cervical screening services in Papua New Guinea. The absence of refusals to participate and lack of study withdrawals suggest a high degree of acceptability of specimen self-collection and are consistent with our previous study in this setting where self-collection was used (A. Vallely, presented at the World STI & HIV Congress 2015, Brisbane, Australia, 13 to 16 September 2015). Previous studies demonstrated that the performance of laboratory-based molecular assays for the detection of HPV infection using self-collected specimens is comparable to that with clinician-collected specimens (18), but none of those studies investigated approaches with the potential for application at the point of care. The strategy evaluated in the present 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 a high overall percent agreement was observed between self-collected vaginal and clinician-collected cervical specimens in the present study, before point-of-care self-collection can be recommended as part of cervical screening algorithms, the performance of Xpert HPV versus 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.

An *a priori* assumption in the present study was that vaginal specimens would be less sensitive for the detection of hrHPV than cervical specimens. Comparison of mean cycle threshold data among concordant paired test results suggests that this is not the case, while the unexpected finding of a 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 (STIs), 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 of 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 precancer lesions is expected to start enrollment in Papua New Guinea in 2016. If self-collection is proven to have performance characteris-

TABLE 2 Summary of disagreements between Xpert HPV test results for vaginal and cervical specimens

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

tics comparable to those of 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.

ACKNOWLEDGMENTS

We thank the women who took part in this research and acknowledge the support of their families and communities, without whom this work would not have been possible. We also thank Cepheid (Sunnyvale, CA) for generously donating Xpert HPV test cartridges for this project.

This work was funded through the National Health and Medical Research Council of Australia and the Papua New Guinea Institute of Medical Research Internal Competitive Research Award Scheme. We also acknowledge the generous core funding support provided to the Papua New Guinea Institute of Medical Research by the Australian Aid Program, Department of Foreign Affairs and Trade, Government of Australia.

None of the funders or other organizations listed had any role in study design, data collection and interpretation, or the decision to submit the work for publication.

FUNDING INFORMATION

This work, including the efforts of A. J. Vallely, was funded by Government of Papua New Guinea (ICRAS 297/1). This work, including the efforts of J. M. Kaldor, was funded by Department of Health | National Health and Medical Research Council (NHMRC) (1071269). This work, including the efforts of A. J. Vallely, was funded by Department of Health | National Health and Medical Research Council (NHMRC) (1013209).

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