Prospective cohort study to evaluate point-of-care HPV-DNA testing for the early detection and treatment of cervical pre-cancer in high-burden, low-resource settings

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ABBREVIATIONS

ASC-US	Atypical squamous cells of undetermined significance
ASC-H	Atypical squamous cells – HSIL cannot be excluded
AIS	Adenocarcinoma in situ
CI	Chief Investigator
CIN	Cervical intraepithelial neoplasia
CRF	Case Report (or Record) Form
CT	*Chlamydia trachomatis*
ENBP	East New Britain Province
HIV	Human Immunodeficiency Virus
HSIL	High grade squamous intraepithelial lesion
HSV 2	Herpes Simplex Virus type 2
GCP	Good Clinical Practice
GCLP	Good Clinical Laboratory Practice
HPV	Human papillomavirus
HSIL	High-grade squamous epithelial lesion
ICER	Incremental cost-effectiveness ratio
IDMC	Independent Data Monitoring Committee
IRB	Institutional Review Board
HREC	Health Research Ethics Committee
LMIC	Low and middle income country
MBP	Milne Bay Province
MP	Madang Province
MRAC	Medical Research Advisory Committee
NAAA / NAAT	Nucleic acid amplification assay / test
NG	*Neisseria gonorrhoeae*
NHMRC	National Health and Medical Research Council
NPV	Negative predictive value
PHA	Provincial Health Authority
PCR	Polymerase chain reaction
pHSIL	Possible HSIL
PI	Principal Investigator
PICT	Provider Initiated HIV Counselling and Testing
PID	Pelvic inflammatory disease
PNG	Papua New Guinea
PNGIMR	Papua New Guinea Institute of Medical Research
POC	Point-of-care
PPV	Positive predictive value
RPR	Rapid plasma reagin
RWH	Royal Women's Hospital
SSI	Semi-structured interview
STI	Sexually Transmitted Infection
SVS	Self-collected vaginal swab
TPHA	*Treponema pallidum* haemagglutination assay
TV	*Trichomonas vaginalis*
UNSW	University of New South Wales, Australia
VIA	Visual inspection of the cervix with acetic acid
WHP	Western Highlands Province
WWC	Well woman clinic

GLOSSARY AND DEFINITIONS

Counsellors	Staff that have undergone nationally recognised training in counselling who may be from a clinical or non-clinical background
Database	The programmed software that will be populated using date captured using electronic CRFs
Datafiles	Data extracted from the database at a given time point
Manual of operating procedures	The manual containing both site specific and trial specific procedures
Negative predictive value (NPV)	The percentage of negative point-of-care test results which are true negatives (as determined by the reference test)
Polymerase chain reaction (PCR)	PCR is used to copy small segments of DNA in organisms which can cause disease such as *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Trichomonas vaginalis*. The method is extremely sensitive and able to pick up very low and very high levels of organism.
Positive predictive value (PPV)	The percentage of positive point-of-care test results which are true positives (as determined by the reference test)
Quality assurance (QA)	Samples with a range of negative and positive levels of non-infectious agent which are periodically supplied to a health service or laboratory by an accredited external proficiency testing provider. Operators are blind to the infectious status of those agents. Results are sent to the external proficiency provider and are subject to a delayed, peer reviewed assessment of analytical quality and accurate record keeping of test results at the service level.
Quality control (QC)	Samples with negative and positive values (high and low titre) of non-infectious agent which are used for regular, routine monitoring of testing performance. Results are available for immediate, internal assessment of analytical quality.
Reference test	The best available laboratory-based method for establishing the presence or absence of an infection
Sensitivity	The percentage of true positives (as determined by the reference test), which are positive on the point-of-care test
Specificity	The percentage of true negatives (as determined by the reference test), which are negative on the point-of-care test
Standard operating procedures	Instructions/flow diagrams to be followed when completing study related tasks
Withdrawal from study	Discontinuation of study visits

PROTOCOL SUMMARY

Title:	**Prospective cohort study to evaluate point-of-care HPV-DNA testing for the early detection and treatment of cervical pre-cancer in high-burden, low-resource settings**
Population:	4000 women aged 30-59 years in Papua New Guinea.
Trial Design:	Non-randomised prospective clinical cohort study.
Research Sites:	Four well woman clinics in East New Britain, Madang, Milne Bay and Western Highlands Provinces.
Study Duration:	3 years.
Subject Duration:	Women will be recruited at their first screening visit and followed for 1 year.
Aim:	To measure the effectiveness, health system implementation requirements, cost-effectiveness and acceptability of point-of-care Xpert HPV testing for the early detection and treatment of cervical pre-cancer in high-burden, low-resource settings.

Research Objectives:

Primary Objective

Evaluate the performance of Xpert HPV Test for the early detection and treatment of cervical pre-cancer lesions when conducted at point-of-care in routine clinical settings using self-collected vaginal specimens.

Secondary Objectives

1. Evaluate the cost-effectiveness of point-of-care Xpert HPV testing for the early detection and treatment of cervical pre-cancer lesions;
2. Evaluate the health system implementation requirements of point-of-care Xpert HPV testing for the early detection and treatment of cervical pre-cancer lesions;
3. Evaluate the acceptability of point-of-care Xpert HPV testing for the early detection and treatment of cervical pre-cancer lesions;
4. Evaluate the laboratory performance of self-collected vaginal specimens compared with clinician-collected cervical specimens for the detection of cervical cancer biomarkers.

1 INTRODUCTION

1.1 Background information

Women in low-resource countries have an extremely high burden of cervical cancer: Of the estimated 528,000 new cervical cancer cases and 266,000 deaths worldwide in 2012, around 90% occurred in developing countries[1,2]. PNG has among the highest estimated burdens of cervical cancer, 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).[1,2] Cervical cancer is the most common cancer among women in PNG and results in an estimated 1,500 deaths per year.[1-3]

Human papillomavirus is the cause of cervical cancer: It has been recognized for several decades that persistent genital human papillomavirus (HPV) infection is the necessary cause of squamous and adenocarcinomas of the cervix, which constitute over 99% of all cervical cancers. There are over 120 types of HPV, around 40 of which infect the anogenital tract through sexual contact.[4] These have been categorized into high-risk or low-risk types based on their ability to cause cervical cancer. Infection with high-risk HPV types (hrHPV) causes the histologically defined condition known as cervical intraepithelial neoplasia (CIN). The highest grade of CIN, designated CIN3, is known to be a 'pre-cancer' lesion, which can progress to invasive cancer if untreated.[5] In high-income settings, the cytology-based Papanicolaou (Pap) test, involving the collection of exfoliated cells from the cervix, has been used to screen for pre-cancer, and led to large reductions in cervical cancer incidence in mortality in many countries, including Australia.[6]

HPV 16 and 18 are the types most commonly associated with both CIN3 and cervical cancer, and estimated to be responsible for around 70% of all cervical cancers worldwide, with types 31, 33, 45, 52, and 58 responsible for an additional 20%.[7] Surveys of HPV prevalence among women in many countries have demonstrated a peak in infection under 25 years followed by a decline with age,[8-10] explained by high incidence in younger women who have recently become sexually active, followed by clearance of infection in the majority of those infected.[8,9] The time lag between first infection and the development of CIN3 can be just a few years[11,12] but progression to cancer is usually several decades.[13] Although a variety of factors increase the likelihood that persistent HPV and CIN3 will progress to cervical cancer[14], including cigarette smoking[15-17] and sexually transmitted infections (STIs),[18-20] it is universally accepted that persistent infection with high risk HPV types is the necessary cause. Our group recently completed the first HPV prevalence surveys in PNG and found high prevalences of infection and biological co-factors associated with disease progression among 1800 women attending well woman, antenatal and sexual health clinics.[21] Around 24% of women aged 30-59 years (n=614) had one or more hrHPV infection and the prevalence of chlamydia, gonorrhoea, trichomonas and herpes simplex virus type-2 (HSV-2) were 5.5%, 6.4%, 11.5% and 59.6% respectively. Members of our group also recently analysed cervical biopsies from 70 women diagnosed with cervical cancer in PNG, and found HPV 16, 18, 33 and 31 the most prevalent high-risk types (57%, 26%, 10% and 4% respectively).[22]

HPV vaccines may protect future generations, but many women already have persistent infection and are at high risk of cancer: Primary prevention of cervical cancer is now a reality with the introduction of safe, high-efficacy prophylactic HPV vaccines administered in early adolescence[23], but their use has been largely restricted to high- and middle-income countries, many of which already have successful Pap screening programs. The availability of subsidized vaccines through the GAVI program has enabled a handful of developing countries to undertake vaccination programs, but for many low-resource countries, including PNG, implementation will be challenging and unlikely to attain substantial coverage in the near future. HPV vaccines do not treat pre-existing HPV infections or influence disease progression among those with HPV-related conditions, such as cervical pre-cancer or cancer. Even where high vaccine coverage can be achieved, there will therefore be no benefit to women who have already acquired persistent hrHPV infection. Cervical screening will hence be required for several decades after the introduction of HPV vaccines.

Inability to identify women with early disease has been a major barrier to cervical cancer control in low-resource countries: A Pap test-based cervical screening initiative for women in PNG was established in 1999 by a non-governmental Australian-supported charity (the MeriPath program) and currently provides a service from more than 30 health facilities in 16 provinces.[24] This program

has been welcome, but its coverage has been very low, with only around 45,000 women having been screened from 2001-2011, or less than 4% of the target population aged 20-59 years. Also, as specimens are sent to Australia for testing, up to half of those needing further investigation or treatment are lost to follow up due to the time between testing and recall. A *Ministerial Task Force on Cervical Cancer* (led by Prof Mola) recently called for alternative, locally-appropriate models of cervical screening and early treatment to be evaluated in PNG. The task force favoured the 'screen and treat' approach endorsed by the World Health Organization (WHO) and based on visualization of the cervix after acetic acid (VIA) plus cervical cryotherapy[25] This strategy involves a speculum examination of the cervix, with naked eye visualization following the application of 5% acetic acid solution, which causes any abnormal areas of cervical epithelium to stain white ('acetowhite staining'). Treatment can then take place on the same day by trained health staff using cervical cryotherapy, in which a specially designed probe connected to a carbon dioxide or nitrous oxide gas cylinder is applied to the cervix and freezes the tissue. Women with lesions that are ineligible for cryotherapy (e.g. too large, in the cervical canal, and/or suspected to be cancer rather than pre-cancer) are referred for specialist medical care e.g. cold knife conization, or large loop excision of the transformation zone using diathermy[26]. On the basis of favourable performance characteristics in research settings (80% sensitivity and 92% specificity for CIN2 or more advanced disease reported in a recent meta-analysis),[27] VIA has been portrayed as an accurate, low-cost screening strategy and implemented in several low-resource countries. Many countries have however experienced difficulties scaling up VIA while maintaining adequate quality and have reported much lower sensitivity and specificity compared to research settings.[28-30]

In PNG, we recently completed the first evaluation of VIA plus cryotherapy for primary cervical screening at two sites (n=614) and found a very poor correlation between VIA and laboratory-based hrHPV test results.[31,32] It was also clear that implementation of VIA was challenging for health staff and medical practitioners, who had difficulties meeting the demand as every patient required an initial pelvic examination. These findings in PNG and elsewhere have stimulated a search for more robust, accurate, reliable and easy-to-use screening methods, particularly those that could be offered at point-of-care, which would enable women at increased risk to be identified and triaged into clinical services.

Testing for HPV-DNA will soon transform cervical screening in high- and middle-income countries globally: The recognition that hrHPV is the primary cause of both cervical pre-cancer and cancer led to the development of new technologies that allow hrHPV DNA to be detected as part of population-based screening. Over the past half-decade, these tests have been shown to be far more sensitive than cytology for the detection of high-grade CIN and invasive disease, to have comparable specificity,[33,34] and their potential efficacy for population-based cervical screening conclusively demonstrated in large-scale randomised trials and prospective studies.[35-37] 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,[34,37-39]. In Australia, members of our group are leading the COMPASS randomised trial comparing 5-yearly hrHPV screening with 2.5-yearly cytology screening for the early detection of CIN3 or cancer. It is the first large-scale trial in the world to assess these screening tests in an HPV-vaccinated population.[38]

In this rapidly moving environment, and based on trials directing comparing HPV screening with cytology,[35-37] WHO recently recommended that hrHPV testing be incorporated into screening programs in low- and middle-income countries where cytological Pap testing is not available.[26] As highlighted by WHO, there was a lack of robust evidence to inform recommendations on the potential role of sequential testing (e.g. hrHPV testing followed by VIA or by cytology) and a need to evaluate different screening options in low-resource settings.

1.2 Scientific rationale

The availability of a new point-of-care hrHPV test could have a potentially game-changing role in developing countries: Although an affordable batch test for the detection of hrHPV infection has recently become available (*care*HPV; Qiagen, Gaithersburg, MD)[40] and performed well in large multi-country demonstration studies[41], the platform uses a 90-well test plate, requires multistep pipetting during processing, and takes 3-4 hours to report results. The platform is therefore unsuitable for low-resource settings as a point-of-care screening test. In contrast, the Xpert HPV

Test (GeneXpert; Cepheid, Sunnyvale, CA) is a newly available, rapid, fully automated and easy to use molecular test for hrHPV infection that is as accurate as laboratory-based nucleic acid amplification (NAA) tests.[34,42] The GeneXpert platform has already transformed the diagnosis and management of tuberculosis in developing countries, including PNG. Disposable cartridges hold the reagents, primers and probes for the simultaneous detection of 14 hrHPV types responsible for over 95% of cervical cancers, and also contain an internal control to verify adequate sample processing. The system monitors the presence of inhibitors in the real-time polymerase chain reaction assay to signal a potentially false negative result. HPV test results are available in 60 minutes.

We have confirmed the excellent laboratory and clinical performance characteristics of Xpert HPV and demonstrated feasibility of use in routine clinic settings in PNG: In earlier research by members of our group, Xpert HPV was shown to have comparable sensitivity, specificity and positive predictive value to the FDA-approved and "gold-standard" cobas 4800 (Roche Molecular Systems, Pleasanton, CA) and Hybrid Capture 2 (hc2; Qiagen, Germantown, MD) PCR assays for the detection of HPV, and high grade cervical intraepithelial neoplasia (CIN).[34,42] For example, the sensitivity of Xpert HPV for CIN grade 2 or above was 90.8% vs. 81.6% for hc2 (p=0.004).[33]

We recently conducted the first evaluation of point-of-care Xpert HPV testing in routine clinical settings in a high-burden, low-resource setting.[43] We demonstrated the feasibility and acceptability of Xpert HPV testing by trained clinical staff in two well woman clinics in PNG (N=1005), where we conducted 18-26 tests per day in each clinic and provided same-day test results to clients. The study demonstrated the excellent performance characteristics of Xpert HPV using self-collected vaginal specimens *vs.* clinician-collected cervical specimens (overall percentage agreement 94.2%; 95%CI: 91.6, 96.9);[43] and evaluated the performance of different clinical algorithms comprising Xpert HPV testing followed by VIA examination.[44] Xpert HPV testing alone was found to be an excellent predictor of underlying HSIL or worse, detected on liquid based cytology (HSIL/HSIL+; sensitivity 91.7%, specificity 87.0%, positive predictive value 34.0%, negative predictive value 99.3%). VIA examination alone had lower performance (51.5%, 81.4%, 17.5%, 95.6% respectively; Table 1). Compared with Xpert HPV testing alone, HPV testing followed by VIA had increased specificity (96.3% vs. 87.0%) but substantially lower sensitivity (45.5% vs. 91.7%) for the detection of HSIL/HSIL+ (Table 2).

Similar findings were reported in two recent studies that evaluated algorithms comprising a combination of laboratory-based HPV testing and VIA examination.[44,45] In both studies, hrHPV testing alone was the best predictor of cervical disease. Sequential testing improved specificity but resulted in substantial loss of sensitivity compared to hrHPV testing alone. In both of these earlier studies, HPV testing was not provided at point-of-care but conducted at a central, off-site laboratory. Initial treatment decisions were therefore made on the basis of VIA examination findings alone, which had low sensitivity and predictive value. Women found to be hrHPV positive were recalled for repeat clinical review and treatment but this resulted in substantial losses to follow-up e.g. in one study, 26% of hrHPV positive women did not return for review.[45] Another recent study attempted to evaluate point-of-care HPV testing using self-sampling followed by VIA but did not ascertain disease status of women who were HPV negative and was therefore unable to fully evaluate the performance of the HPV-based screening algorithm used.[46]

Following these findings and the results of our earlier research, we now propose a large-scale field evaluation of the effectiveness, health system implementation requirements, cost-effectiveness and acceptability of point-of-care Xpert HPV testing for the early detection and treatment of cervical pre-cancer in PNG and other high-burden, low-resource settings.

Table 1. Performance of VIA examination alone or Xpert HPV Test alone for the detection of high-grade disease (HSIL/HSIL+)

		High-grade disease		Performance (95% CI)
		POS n (%)	NEG n (%)	
VIA only (n=462)	POSITIVE n=97	17 (3.7)	80 (17.3)	SENS: 51.5 (33.5-69.2) SPEC: 81.4 (77.3-84.9) PPV: 17.5 (10.6-26.6) NPV: 95.6 (93.0-97.5)
	NEGATIVE n=365	16 (3.5)	349 (75.5)	
Any hrHPV self-collected vaginal specimen (n=527)	POSITIVE n=97	33 (6.3)	64 (12.1)	SENS: 91.7 (77.5-98.2) SPEC: 87.0 (83.7-89.8) PPV: 34.0 (24.7-44.3) NPV: 99.3 (98.0-99.9)
	NEGATIVE n=430	3 (0.6)	427 (81.0)	
Any hrHPV clinician-collected cervical specimen (n=529)	POSITIVE n=81	33 (6.2)	48 (9.1)	SENS: 91.7 (77.5-98.2) SPEC: 90.3 (87.3-92.7) PPV: 40.7 (29.9-52.2) NPV: 99.3 (98.1-99.9)
	NEGATIVE n=448	3 (0.6)	445 (84.1)	

Table 2. Performance of different clinical screening algorithms comprising Xpert HPV Test conducted at POC using self-collected vaginal specimens and VIA examination for the detection of high-grade disease (HSIL or worse)[44]

Screening Algorithm		High-Grade Disease		Percentage %			
		POS n (%)	NEG n (%)	SENS (95% CI)	SPEC (95% CI)	PPV (95% CI)	NPV (95% CI)
POSITIVE n=33	Any hrHPV+/VIA+	15	18	83.3 (58.6-96.4)	96.0 (93.7-97.6)	45.5 (28.1-63.6)	99.3 (98.0-99.9)
NEGATIVE n=430	Any hrHPV-	3	427				
POSITIVE n=33	Any hrHPV+/VIA+	15	18	45.5 (28.1-63.6)	96.3 (94.2-97.8)	45.5 (28.1-63.6)	96.3 (94.2-97.8)
NEGATIVE n=482	Any hrHPV- OR Any hrHPV+/VIA-	18	464				
POSITIVE n=25	HPV16+	14	11	82.4 (56.6-96.2)	97.5 (95.6-98.7)	56.0 (34.9-75.6)	99.3 (98.0-99.9)
NEGATIVE n=430	Any hrHPV-	3	427				
POSITIVE n=16	HPV16+/VIA+	11	5	78.6 (49.2-95.3)	98.8 (97.3-99.6)	68.8 (41.3-89.0)	99.3 (98.0-99.9)
NEGATIVE n=430	Any hrHPV-	3	427				
POSITIVE n=16	HPV16+/VIA+	11	5	64.7 (38.3-85.8)	98.9 (97.4-99.6)	68.8 (41.3-89.0)	98.6 (97.0-99.5)
NEGATIVE n=438	Any hrHPV- OR HPV16+/VIA-	6	432				
POSITIVE n=33	HPV16/18/45+	19	14	86.4 (65.1-97.1)	96.8 (94.7-98.3)	57.6 (39.2-74.5)	99.3 (98.0-99.9)
NEGATIVE n=430	Any hrHPV-	3	427				
POSITIVE n=18	HPV16/18/45+/VIA+	13	5	81.3 (54.4-96.0)	98.9 (97.4-99.6)	72.2 (46.5-90.3)	99.3 (98.0-99.9)
NEGATIVE n=430	Any hrHPV-	3	427				
POSITIVE n=18	HPV16/18/45+/VIA+	13	5	61.9 (38.4-81.9)	98.9 (97.4-99.6)	72.2 (46.5-90.3)	98.2 (96.5-99.2)
NEGATIVE n=443	Any hrHPV- OR HPV16/18/45+/VIA-	8	435				
POSITIVE n=10	HPV18/45+	6	4	66.7 (29.9-92.5)	99.1 (97.6-99.7)	60.0 (26.2-87.8)	99.3 (98.0-99.9)
NEGATIVE n=430	Any hrHPV-	3	427				
POSITIVE n=3	HPV18/45+/VIA+	2	1	40.0 (5.3-85.3)	99.8 (98.7-100)	66.7 (9.4-99.2)	99.3 (98.0-99.9)
NEGATIVE n=430	Any hrHPV-	3	427				
POSITIVE n=3	HPV18/45+/VIA+	2	1	25.0 (3.1-65.0)	98.6 (97.0-99.5)	66.7 (9.4-99.2)	99.8 98.7-100)
NEGATIVE n=434	Any hrHPV- OR HPV18/45+/VIA-	6	430				

2 OBJECTIVES AND OUTCOME MEASURES

The overall aim of this study is to measure the effectiveness, health system implementation requirements, cost-effectiveness and acceptability of point-of-care HPV-DNA testing for the early detection and treatment of cervical pre-cancer in high-burden, low-resource settings.

2.1 Objectives

Primary Objective

Evaluate the performance of Xpert HPV Test for the early detection and treatment of cervical pre-cancer lesions when conducted at point-of-care in routine clinical settings using self-collected vaginal specimens.

Secondary Objectives

1. Evaluate the cost-effectiveness of point-of-care Xpert HPV testing for the early detection and treatment of cervical pre-cancer lesions;
2. Evaluate the health system implementation requirements of point-of-care Xpert HPV testing for the early detection and treatment of cervical pre-cancer lesions;
3. Evaluate the acceptability of point-of-care Xpert HPV testing for the early detection and treatment of cervical pre-cancer lesions;
4. Evaluate the laboratory performance of self-collected vaginal specimens for the detection of cervical cancer biomarkers compared with clinician-collected cervical specimens.

2.2 Outcome measures

Primary Outcome Measure

Performance (sensitivity, specificity, positive and negative predictive value) of Xpert HPV Test conducted at point-of-care using self-collected specimens to detect and treat cervical pre-cancer.

- We have selected liquid based cytology (LBC) as the most appropriate reference standard against which to evaluate our novel screening algorithm, and will use the cytological finding of high-grade squamous intraepithelial lesion (HSIL) as our disease threshold.
- Colposcopy and biopsy for a histological diagnosis would be the preferred standard in a high-income country but is not considered feasible or sustainable for health service delivery in this setting.
- Liquid based cytology (LBC) will be carried out at the Victorian Cytology Service (VCS), Melbourne, under the direction of A/Prof Saville. In the Australian setting, cytology has a positive predictive value of around 80% for CIN2 or CIN3. It is anticipated that the positive predictive value of cytology will be greater in PNG because of the expected higher prevalence of CIN2 in this largely unscreened population.
- In order to further strengthen the predictive value of LBC:

  (a) all specimens will be read independently by two teams consisting of scientists and pathologists at VCS who are blinded to the Xpert HPV test result and the other team's assessment;

  (b) where both teams have called a case HSIL (or adenocarcinoma in situ; AIS) no further investigation is required and a final diagnosis of HSIL / high-grade disease will be made;

  (c) in cases where the assessment of each team differs and one team assess the case to be possible HSIL (pHSIL) or worse, dual p16/Ki-67 immunostaining will be carried out using the Roche CINTec® PLUS Cytology test in order to make a final diagnosis (Figure 1).

- We will calculate sensitivity, specificity, positive and negative predictive values (with 95% confidence intervals) of the Xpert HPV Test to detect high-grade disease and calculate the proportion of all those with disease who receive ablative treatment at point-of-care.
- The performance of Xpert HPV under different scenarios will also be calculated (e.g. any hrHPV+; HPV16+; HPV 16/18/45+; hrHPV+ at different PCR cycle thresholds [a proxy for HPV viral load]; hrHPV and biomarker assay combinations).

Figure 1: Decision matrix for cytology specimens

		Team A					
		Unsat	Neg	pLSIL (ASC-US)	LSIL	pHSIL (ASC-H) glandular abnormality less than AIS	HSIL / AIS
Team B	Unsat	Unsat	Not HSIL	Not HSIL	Not HSIL	Dual stain, consider HSIL if positive	Dual stain, consider HSIL if positive
	Neg	Not HSIL	Not HSIL	Not HSIL	Not HSIL	Dual stain, consider HSIL if positive	Dual stain, consider HSIL if positive
	pLSIL (ASC-US)	Not HSIL	Not HSIL	Not HSIL	Not HSIL	Dual stain, consider HSIL if positive	Dual stain, consider HSIL if positive
	LSIL	Not HSIL	Not HSIL	Not HSIL	Not HSIL	Dual stain, consider HSIL if positive	Dual stain, consider HSIL if positive
	pHSIL (ASC-H) glandular abnormality less than AIS	Dual stain, consider HSIL if positive	Dual stain, consider HSIL if positive	Dual stain, consider HSIL if positive	Dual stain, consider HSIL if positive	Dual stain, consider HSIL if positive	Dual stain, consider HSIL if positive
	HSIL / AIS	Dual stain, consider HSIL if positive	Dual stain, consider HSIL if positive	Dual stain, consider HSIL if positive	Dual stain, consider HSIL if positive	Dual stain, consider HSIL if positive	**HSIL**

HSIL: high-grade squamous epithelial lesion
pHSIL: possible HSIL
ASC-US: atypical squamous cells of undetermined significance
ASC-H: atypical squamous cells – HSIL cannot be excluded
AIS: adenocarcinoma in situ

Secondary Outcome Measures

1. Cost-effectiveness

Cost-effectiveness of point-of-care HPV testing will be evaluated using methods used by A/Prof Canfell and Dr Simms for evaluation of similar screening programs in Australia, and in low- and middle-income countries (LMICs). Briefly, the impact of different scenarios on HPV and cervical cancer will be modelled. This will include scenarios specific to this trial and to PNG (described further under health system implementation requirements below) and screening scenarios recommended by the American Society for Clinical Oncology for resource limited settings.[47]

Incremental cost-effectiveness ratios (ICERs) for each scenario will be calculated for the detection and treatment of HSIL using cost data sourced from health facilities, and health service clients. Long term outcomes will also be modelled as cost per life year saved and cost per DALY averted.

- Standard cost-effectiveness analyses will be performed by identifying the cost-effectiveness frontier and calculating the incremental cost-effectiveness ratios (ICERs) of strategies on the frontier compared to the next most cost-effective strategy.
- We will calculate all cost-effectiveness ratios as cost per life year saved. A 3% discount rate will be used for both costs and benefits. The gross regional product (GRP) per capita for the relevant province will be used as the cost-effectiveness threshold for the evaluation. Formally, interventions with ICERs less than this value are considered “very cost-effective,” according to WHO guidelines.

- In addition to cost-effectiveness outcomes, we will present health outcome estimates (number of new cervical cancer cases and cervical cancer deaths per year), resource utilisation estimates (including number of tests taken and number of treatments for precancer) and estimates of the cost per year for cervical screening, which can be used to inform budget impact of a national cervical screening program.
- Extensive sensitivity analysis of the findings will be performed, using one-way and probabilistic sensitivity analysis approaches to assess the effect of variation in model assumptions on the health economic outcomes.

2. Health system implementation requirements

Health system implementation requirements for point-of-care Xpert HPV testing will be evaluated at the clinic level in comparison to (i) current screening practice (VIA or Pap test); (ii) no screening but with attendance at a well woman clinic. The benefits and challenges of HPV-based screening will also be assessed. This will involve collection and analysis of:

a) Quantitative data - using structured observation and facility records review as tools - on patient-flow and turnaround-time (i.e. time from arrival at clinic to Xpert tests result and treatment if positive); work-flow and staff time; infrastructure, logistics and management and changes in services delivered at well woman clinics.

These data will be combined with costing data derived from trial documentation to capture the complete costs of the intervention; for use in cost-effectiveness calculations, and in establishment of the complete envelope of staff, infrastructure, commodity and additional monetary (e.g. incentives) resources required for (i) introduction and (ii) maintenance of Xpert HPV testing.

We will be flexible in order to measure requirements for different clinical algorithms in different clinics, particular in relation to hrHPV positive women. We will also take a modular approach i.e. measure requirements for each step in the algorithm separately. This will provide data which can be used in predicting resource needs for point of care HPV screening programs which might be adapted for different settings or modes of delivery in future, for example via community outreach or in lower level clinics;

b) Qualitative data on resource needs, training, supervision, logistics, shifts in client-provider relationships when moving from current routine screening (based on VIA alone or Pap test) to HPV-based screening, changes in service provision and other aspects of operational feasibility will be collected in semi-structured interview (SSI) and focus group discussions (FGDs) with clinicians, clinic managers, laboratory staff and other key local stakeholders.

c) Qualitative data on issues concerning scaling up and sustainability of point of care HPV screening beyond the study will be collected in semi-structured interviews at the end of study with key local and national-level stakeholders.

3. Acceptability

Acceptability of the intervention from client and health provider (clinical staff and health managers) perspectives.

- Quantitative data will be collected from study participants at enrolment and clinical follow-up visits using a study-specific CRF administered by research staff. This will measure satisfaction with services in all sites and evaluate acceptability and client opinions regarding the intervention. We will calculate the proportion of respondents who consider the intervention ‘acceptable’ or ‘highly acceptable’ (overall and by individual clinic site), including assessment of whether they would recommend the screening to other women.
- We will also collect data on the number of women offered HPV testing who declined to enrol in the study, and their reasons for not wishing to participate.
- Qualitative data will be collected from study participants, clinic staff, health managers, and other key stakeholders in the pre-intervention and intervention phases of the study in focus group discussions (FGDs) and semi-structured interviews (SSIs) that will investigate women’s preferences for service delivery in relation to HPV screening, and the wider societal and cultural contexts within which the acceptability of new interventions for cervical cancer are framed and perceived. Data collection from staff, managers and key stakeholders will be coordinated with health service implementation procedures.

4. Cervical cancer biomarkers

Laboratory performance characteristics (sensitivity, specificity, positive and negative predictive value) of self-collected compared with clinician-collected specimens for the detection of cervical cancer biomarkers.

Residual self-collected and clinician-collected specimens in ThinPrep PreservCyt (Hologic, Marlborough, MA) will be tested at the Labnet HPV Regional Reference Laboratory at the Royal Women's Hospital, Melbourne, and/or at the Victorian Cytology Service, Melbourne for:

- HPV16 and HPV18 E6 oncoprotein using the Onco***E6***™ Cervical Test (ArborVita Corp., CA);
- HPV-transformed cells using the CINTec® PLUS (Roche) p16/Ki-67 dual stain assay;
- Methylation analysis of CADM1, MAL, and miR124-2 genes e.g. using the PreCursor-M assay (Self-Screen BV, Amsterdam).[48,49]

3 STUDY DESIGN

3.1 Overview

A prospective clinical cohort study will be conducted among 4000 women aged 30 – 59 years attending Well Woman Clinics in Papua New Guinea.

3.2 Rationale

We have adopted a non-randomised longitudinal prospective cohort study design as our preferred option. A randomised design (e.g. Xpert HPV at point-of-care compared with VIA alone or VIA plus Xpert HPV testing) was not considered ethically feasible due to the poor performance of VIA-based screening algorithms in this setting, as demonstrated in our earlier research (Table 1, 2).

In order to evaluate health economics, health systems and acceptability outcomes in depth, we have included a preparatory, **pre-intervention phase** in our study design that will allow us to collect data before and after implementation of HPV point-of-care testing in each clinical setting.

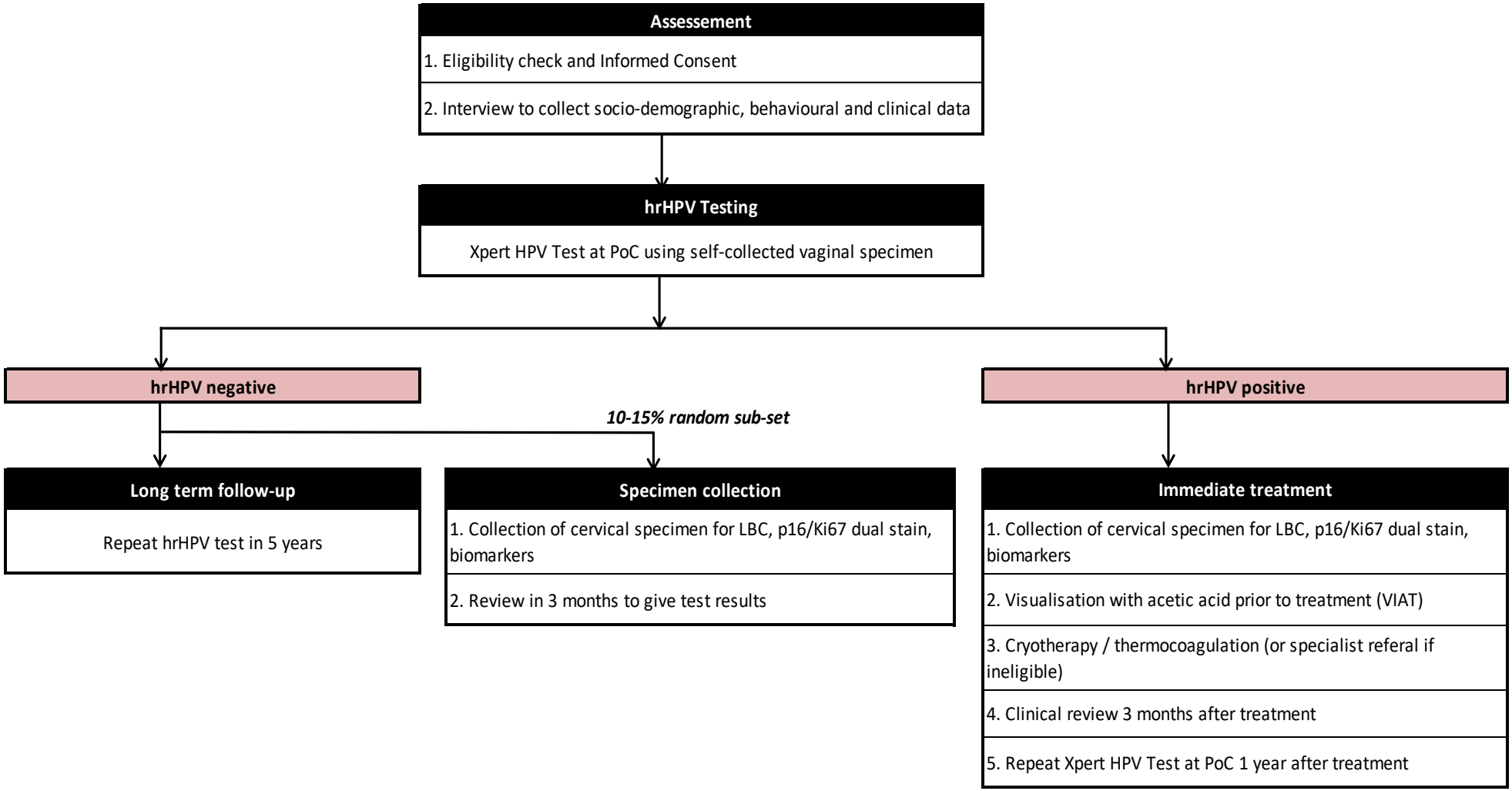
3.3 Intervention summary

Women will provide self-collected vaginal specimens for point-of-care Xpert HPV testing that will be carried out in the clinic and results provide the same day. Subsequent clinical management and scheduled follow-up will be determined by their Xpert HPV test result (Figure 2):

1) **Women who are hrHPV positive** will provide additional specimens for laboratory investigation and undergo clinical examination prior to treatment:
   - A cervical cytobrush specimen will be obtained and placed in 20 ml PreservCyt. This specimen will not be tested onsite but will be stored at 4^{o}C prior to shipment to Melbourne for liquid based cytology and p16/Ki67 dual stain testing. Residual specimens will be tested for cervical cancer biomarkers (described above).
   - Visualisation of the cervix with acetic acid prior to treatment (VIAT) will then be carried out:
     - If the transformation zone (TZ) is visible in its entirety and occupies an area smaller than the largest treatment probe available in the clinic, the woman will be offered same-day ablative cervical cryotherapy or thermocoagulation as per study-specific standard operating procedures.
     - If the transformation zone appears too large to be treated using clinic-based instruments and/or another abnormality is present on inspection (e.g. suspected cervical cancer; cervical polyp), the woman will be referred for specialist care at the participating hospital.
   - Women will be asked to return for clinical review at 3 months to confirm resolution post-treatment, as per WHO guidelines.
   - A repeat Xpert HPV test will be conducted at 1 year to confirm cure. If the repeat HPV test is positive, VIAT and cervical ablation will be repeated as described above.

2) **Women who are hrHPV negative** will be discharged from the study and advised to return to the clinic in 5 years for repeat HPV-based cervical screening in accordance with current international guidance.[35,50]
   - A 15% randomly selected sub-population of Xpert HPV negative women will be asked to provide cervical specimens for liquid based cytology, p16/Ki67 dual stain testing; and cervical cancer biomarkers; and asked to return to the clinic to receive for their test results in 3 months.

Figure 2. Clinical screening algorithm



4 STUDY POPULATION

4.1 Selection of study sites

Study sites were selected in consultation with national and provincial health authorities, church health services, health facility staff and local stakeholders in each province.

Criteria for study site selection included: experienced consultant obstetrician/gynaecologist on-site and available to support the research, including review of referral cases; dedicated Well Woman Clinic (WWC) operating on at least three days per week providing Pap test or VIA-based cervical screening; suitable clinic space available for setup of new equipment, conduct of clinical interviews and other research activities; clinical workload of >200 women per month; and previous experience in PNGIMR-led collaborative research projects (particularly those evaluating cervical screening algorithms and/or which previously used GeneXpert diagnostic tests).

Table 3: Selected study sites

East New Britain Province	Madang Province	Milne Bay Province	Western Highlands Province
WWC, St Mary's Vunapope	WWC, Modilon Hospital	WWC, Alotau Provincial Hospital	WWC, Mt Hagen General Hospital

Memoranda of Agreement between the PNGIMR and provincial health authorities in each of the above provinces will be established prior to the start of the study and provide an administrative framework for the study at local level.

4.2 Eligibility criteria

- Aged 30-59 years attending a participating Well Woman Clinic;
- Willing to provide self-collected vaginal swabs for baseline Xpert HPV testing and to comply with study follow-up procedures;
- Willing to undergo a clinical interview and pelvic examination, and to provide self-collected and clinician-collected specimens for laboratory investigations;
- Able to complete study informed consent procedures; to understand why the study is being carried out, and the potential risks and benefits associated with study participation;
- Able to provide reliable contact details to facilitate future community tracing and follow-up.

Eligibility criteria will be assessed at the start of the clinic visit as part of study informed consent procedures.

4.3 Exclusion criteria

The following exclusion criteria apply:

- Currently pregnant or given birth in the last 6 weeks;
- Previous diagnosis of cervical cancer and/or has had a hysterectomy;
- Permanent disability, that prevents or impedes study participation and/or comprehension (such that it is not possible to obtain informed consent to participate);
- Women who are having their menstrual period at the time of the clinic visit will be advised to return for screening in 1-2 weeks.

Exclusion criteria will be assessed at the start of the clinic visit as part of study informed consent procedures. All those excluded on health grounds will be referred to the appropriate clinical specialist at each site, where they will be treated according to PNG national treatment guidelines.[51]

5 STUDY PROCEDURES

5.1 Field team

5.1.1 Roles and responsibilities

Overall responsibility for the conduct and management of the study will rest with the investigator team. The study will be managed on a day-to-day basis by a Study Coordinator based at the PNGIMR who will oversee the study at all sites and supervise a dedicated field team comprising clinical, laboratory, data management, community liaison and administrative support staff.

Table 4. Field team roles and responsibilities

Role	Responsibilities
Study Coordinator (1.0)	Coordinate and manage the study at all sites on a day-to-day basis.
Laboratory Quality Control Manager (0.20)	Laboratory quality monitoring, quality control and quality assurance of all clinic and laboratory-based assays conducted in the trial.
Laboratory Technician (0.20)	Training and support supervision of clinical staff conducting point-of-care testing; laboratory logistics and coordination.
Research Nurse (6.0; 1-2 staff per clinic)	Conduct participant recruitment and clinic-based follow-up, including informed consent, point-of-care Xpert HPV testing, clinical interview and examination, as specified in the study protocol and Standard Operating Procedures (SOPs)
Health Systems Researcher (0.30)	Conduct interviews with key informants and stakeholders, conduct structured observation, and analyse facility and trial records, for health economics and health systems outcomes of the trial.
Qualitative Researcher (0.60)	Conduct interviews with a subset of trial participants to capture acceptability outcome data.
Data Manager (1.0)	Management of tablet-based electronic data capture using electronic CRFs (eCRFs)
Community Tracer / Village Reporter (6.0)	Conduct site-level community liaison activities; participant tracing.
Driver (3.0)	Transport of clinic and community teams, vehicle maintenance and safety.

Each member of the research team will sign the study Delegation Log describing their role and responsibility within the team and specifying which procedures they are authorised by the investigator team to conduct as part of the study. Team members will also be asked to sign a Code of Research Conduct confirming that they will conduct their duties in accordance with the approval study protocol, study-specific standard operating procedures (SOPs), ICH GCP and relevant national and international ethics committee guidelines and requirements.

5.1.2 Training in research ethics and study procedures

Dr Vallely will be responsible for providing induction and refresher training in research ethics and ICH Good Clinical Practice Guidelines (GCP) to study staff. All staff will be required to complete a basic training program comprising an introduction to clinical research ethics, study aims and objectives, study schedule, procedures, anticipated outcomes and scientific significance.

Clinical research staff will receive additional training according to their designated roles and responsibilities as specified in the trial Responsibilities Log and in accordance with study-specific SOPs and guidelines. Staff responsible for operation of the GeneXpert machine will be required to complete a formal competency assessment and/or external accreditation before being permitted to conduct these investigations as part of the trial.

Community-based research staff will take part in site-level workshops to finalise study-specific community SOPs that will guide community liaison and participant tracing activities during the trial.

5.2 Community mobilisation and liaison

Community-based networks established in our earlier studies will be used to inform potential participants in clinic catchment areas and encourage them to attend study clinics.

Immediately prior to the start of enrolment at each site, potential participants will be notified that the trial is about to start through community meetings (e.g. at markets, after church), local media (e.g. broadcasts in PNG Pidgin and local languages), and other mobilisation activities as advised by local stakeholders. Study-specific SOPs will be developed to guide the implementation and monitoring of community activities at each site.

5.3 Informed Consent

5.3.1 First contact and pre-enrolment eligibility assessment

Women will be provided with general information about the study on arrival at the clinic though a 5-10 minute group talk given by a member of the clinical research team (referred to as a '*tok save*' in PNG Pidgin):

- The talk will cover key study objectives and procedures; eligibility and inclusion/exclusion criteria; and the benefits and potential risks of study participation.
- A pictorial flipchart will be used to supplement the talk and to explain key procedures in more detail, such as the collection of genital specimens and treatment options. The format and content of the flipchart will be informed by our earlier work in this setting.[43,44,52]
- Staff will also have examples of unused specimen collection kits available for women to look at and handle for themselves.
- At the end of the talk, copies of the study Participant Information Sheet will be provided.

Women who are willing to join the study will be asked to complete a short pre-enrolment eligibility assessment conducted by a member of the research team:

- If she is eligible to participate, formal informed consent procedures will then be completed in accordance with study-specific SOPs.
- If she is not eligible, the reasons she cannot join the study will be explained to her, and her Eligibility Form filed in the Exclusions Register.

Women who choose not to participate in the study or are ineligible for reasons as stated above will be assured of receiving standard care as per current PNG standard treatment guidelines.[51]

5.3.2 Written informed consent procedures

Women who have completed the above procedures will be asked to complete formal written informed consent procedures that will comprise:

- A face-to-face discussion with a member of the clinical research team who will explain key study objectives, procedures, potential risks and benefits in more detail; including that participation is entirely voluntary, withdrawal is possible at any time without having to give a reason, and that refusal to participate or withdrawal from the trial will not affect a woman's current or future access to routine health care in any way.
- A short Comprehension Checklist completed by study staff to confirm that each participant understands key aspects of the study prior to enrolment.
- Signing of the Informed Consent Form by the study participant and the staff member obtaining consent.

In accordance with ICH GCP, participants who are unable to read and/or write will be required to have an impartial witness present during the above procedures in order to ensure that there is no risk of misunderstandings or possible coercion. In such cases, participants will provide a thumbprint indicating their consent to participate, which will be verified by the witness who will sign and date the consent form.

In order to identify women who require a witness as soon as possible in the formal consent process, at the start of written consent procedures all women will be asked to complete a Literacy Check Form, comprising a simple example sentence in English and PNG Pidgin (to be read aloud) and a space for participants to write their full name and date of birth.

All participants will be given a copy of the Participant Information Sheet to take home.

5.4 Enrolment visit procedures

Following the completion of written informed consent procedures, participant details will be entered into the Study Register and she will be allocated a unique alphanumeric Study Identification (ID) Number that will be used to identify her clinical and laboratory information in paper-based and electronic records to ensure confidentiality. This number will also be recorded on the inside cover of her client-held Health Record Book. If she does not have her own Health Record Book, the team will provide her with one free-of-charge at enrolment.

The study enrolment visit will comprise the following:

- A short face-to-face clinical interview conducted in the most appropriate language (e.g. English or PNG Pidgin): to collect baseline socio-demographic, behavioural and clinical information.
- Xpert HPV Test using a self-collected vaginal cytobrush specimen: clinic staff will use a laminated pictorial guide to help them explain the correct way to collect vaginal specimens for testing. During the explanation, staff will indicate how samples are to be collected using a specimen collection kit reserved for this purpose, and will encourage women to handle cytobrushes, and to review the pictorial guide themselves.
- When staff are satisfied that the participant understands the collection procedure, she will be asked to collect her specimens in a private room or the clinic toilet. This approach has been successfully used in our earlier research and found to be acceptable to both study participants and clinic staff.
- Self-collected specimens will be returned to clinic staff and immediately placed in 20 ml ThinPrep PreservCyt (Hologic, Marlborough, MA) prior to testing for hrHPV on the GeneXpert platform which will be conducted in accordance with manufacturer's instructions and study-specific SOPs.
- Residual self-collected PreservCyt specimens will be retained and stored at 4^{o}C prior to shipment to Melbourne for p16/Ki67 dual stain testing and cervical cancer biomarkers.

Further management will be determined by the Xpert HPV Test result (Figure 2)

1) **Women who are hrHPV positive** will provide additional specimens for laboratory investigation and undergo clinical examination prior to treatment:
   - A cervical cytobrush specimen will be obtained and placed in 20 ml PreservCyt. This specimen will not be tested onsite but will be stored at 4^{o}C prior to shipment to Melbourne for liquid based cytology and p16/Ki67 dual stain testing; and cervical cancer biomarkers.
   - Visualisation of the cervix with acetic acid prior to treatment (VIAT) will then be carried out:
     - If the transformation zone is visible in its entirety and occupies an area smaller than the largest treatment probe available in the clinic, the woman will be offered same-day ablative cervical cryotherapy or thermocoagulation, as per study-specific SOPs.
     - If the transformation zone appears too large to be treated using clinic-based instruments and/or another abnormality is present (e.g. suspected cervical cancer; cervical polyp), the woman will be referred for specialist care.
     - Clinicians will also record examination findings including acetowhite staining following acetic acid application.
   - Women will be asked to return for clinical review at 3 months to confirm resolution post-treatment, as per WHO guidelines.
   - A repeat Xpert HPV test will be conducted at 1 year to confirm cure. If the repeat HPV test is positive, VIAT and cervical ablation will be repeated and carried out as described above.
   - The outcome of specialist referrals will be discussed with the attending gynaecologist and a final diagnosis recorded in a study-specific electronic CRF via a tablet (e.g. iPad or similar).

3) **Women who are hrHPV negative** will be discharged from the study and advised to return to the clinic in 5 years for repeat HPV-based cervical screening, in accordance with current international guidance.[35,50]
   - A 15% randomly selected sub-population of Xpert HPV-negative women will be asked to provide cervical specimens for liquid based cytology, p16/Ki67 dual stain testing; and

cervical cancer biomarkers; and to return to the clinic to receive test results in 3 months.

- The pre-printed Study Register will indicate whether a woman has been randomly selected to participate in this part of the study (e.g. a symbol adjacent to the Study Number in the register). In the situation where an indicator is present in the register but the participant has a positive Xpert HPV Test result, staff will enrol the next HPV negative woman.

In the event of an invalid Xpert HPV Test result being obtained

- Women will be asked to provide an additional self-collected swab and given further counselling on self-collection, to enable a re-test to be carried out the same day in the clinic.

Routine clinic procedures

In addition to the study-specific procedures described above, all women will be offered routine care in accordance with PNG national guidelines, including syndromic STI management.[51]

Collection of locator information

- At the end of the enrolment visit, a short interview with a dedicated community tracer will be conducted in order to collect home address and other locator information to facilitate future community tracing and follow-up.
- A separate Locator Form will be used to record this information and will be securely stored in a different location to participant clinical information e.g. laboratory test logs.

5.5. Procedures during clinical follow-up

5.5.1. Procedures at three-month follow-up visit

Women who test Xpert HPV positive at enrolment and a randomly selected sub-population of women who test Xpert HPV negative at enrolment will be asked to return for clinical review three-months after enrolment (Table 5).

Table 5. Summary of study procedures at three-month clinical follow-up visit

	Xpert HPV+ at enrolment (treated at enrolment or referred for specialist review)	**Xpert HPV- at enrolment** (randomly selected to provide additional specimens)
Clinical interview	Yes – to capture acceptability, behavioural and clinical data using a study-specific electronic CRF	Yes – to capture acceptability data using a study-specific electronic CRF
Pelvic examination	Yes – to confirm resolution following cervical ablative therapy	No - unless high grade lesion or worse detected on LBC
Provided with baseline LBC / p16/Ki67 and biomarker test results	Yes	Yes
Further management	Next scheduled follow-up **12 months** after enrolment for repeat HPV test; earlier if clinically indicated e.g. positive LBC / p16/Ki67 or biomarker test results	Next scheduled follow-up **5 years** after enrolment for repeat HPV test; earlier if clinically indicated e.g. positive LBC / p16/Ki67 or biomarker test results

5.5.2. Procedures at 12-month follow-up visit

Women who test Xpert HPV positive at enrolment will be asked to return for clinical review at 12-months after enrolment, when they will be asked to complete the following study procedures:

- A clinical interview to capture acceptability, behavioural and clinical data, recorded using a study-specific electronic CRF.
- Repeat Xpert HPV Test using a self-collected cytobrush specimen (as described above):
  - Women who have a **negative Xpert HPV Test** at 12-months will be advised that their HPV infection and associated cervical disease have been successfully treated, and that they should return for repeat HPV-based screening in 5 years.
  - Women who have a **positive Xpert HPV Test** at 12-months will be advised to have a repeat pelvic examination and same day ablative treatment or referred for specialist review if indicated on pelvic examination findings.

5.6 Procedures to maximise cohort retention

We will use procedures established in our earlier research in this setting to achieve high retention in clinical follow-up. Dedicated community tracers / village reporters will collect detailed contact information at the first clinic visit and visit each participant in the community to confirm their home address. Tracers will be allocated specific communities within their clinic catchment area to facilitate travel logistics and to enable them to develop an in-depth understanding of community-specific geography and structure.

At the first home visit, tracers will record walking tracks, major landmarks and other information required to successfully re-locate the residence in future, and summarise these data in a simple hand-drawn map, following procedures established in our earlier research. Locator information will be checked at each clinic visit and any changes noted.

Women who do not attend scheduled follow-up visits will be traced in the community and asked to return for review.

5.7 Laboratory procedures

5.7.1 Xpert HPV Test conducted at point-of-care

The Study Coordinator will have overall responsibility for the conduct of point-of-care testing at each clinic site.

- Xpert test cartridges, cytobrush packaging and PreservCyt tubes will all be pre-labelled with both the participant's Study ID Number and a unique Laboratory Sticker Number that will contain a bar code which will be scanned prior to Xpert testing.
- Immediately following specimen collection, participants will hand their self-collected cytobrush specimens (placed back in the original packaging) directly to the research nurse for processing and testing.
- Specimen handling and testing SOPs have previously been developed in our earlier research in Australia and PNG. A laminated Quick Reference Guide will be packed into the GeneXpert case and placed in the testing area on each clinic day for ease of reference.
- Xpert point-of-care testing will be conducted on the morning of each clinic day in order to provide results to participants the same day and to allow the afternoon sessions at each clinic to be devoted to clinical management of HPV+ women.
- Xpert HPV test results will be recorded by Study ID and Laboratory Sticker Number in a Test Results Log, and will automatically be stored by Laboratory Sticker Number on the GeneXpert-associated laptop computer. At the end of each clinic day, the electronic test results database will be backed-up on the computer and on a removable USB drive as per study-specific SOPs.

5.7.2 Laboratory tests conducted off-site

The following tests will be conducted at the Victorian Cytology Service (VCS), Melbourne, and the Labnet HPV Regional Reference Laboratory, Royal Women's Hospital (RWH), Melbourne using residual self-collected and clinician-collected PreservCyt specimens:

- Liquid based cytology and p16/Ki-67 dual stain (using the CINTec® PLUS (Roche) assay;
- HPV16 and HPV18 E6 oncoprotein using the Onco***E6***™ Cervical Test (ArborVita Corp., CA);
- HPV-transformed cells using the CINTec® PLUS (Roche) p16/Ki-67 dual stain assay;
- Methylation analysis of CADM1, MAL, and miR124-2 genes e.g. using the PreCursor-M assay (Self-Screen BV, Amsterdam).[48,49]

5.7.3 Quality Control and Quality Assurance

The study will be conducted in accordance with WHO Good Clinical Laboratory Practice guidelines (GCLP).[53] Procedures for the collection, storage documentation and reporting of laboratory data will be GCLP compliant.

- Standard Operating Procedures for quality control and assurance of all assays used in the study will be established and approved by a Laboratory Working Group comprising senior trial investigators and field staff.
- Study-specific SOPs will be developed on a number of topics including: test storage conditions; the collection of samples; infection control; use and maintenance of the Xpert system; quality

management processes; recording of test results; and on-site and off-site secure electronic test data back-up.

- A dedicated member of the investigator team will be responsible for coordination and management of laboratory quality control and assurance and will report directly to the CI, Study Coordinator and the Laboratory Working Group.
- Clinical research staff will receive training in the conduct of all assays to be conducted at point-of-care and the quality control and assurance measures to be followed. Staff competency will be assessed by the Laboratory QC/QA Coordinator before staff are authorised to conduct tests. Only staff who have successfully completed competency training and have signed the study Delegation Log will be permitted to carry out these tests.

In regards quality control and assurance procedures to be followed for the Xpert HPV Test the following principles will apply:

- The GeneXpert system has a range of sophisticated in-built quality test processes including a Sample Processing Control (SPC), a Sample Adequacy Control (SAQ) and a Probe Check Control (PCC).
- Each Xpert cartridge contains internal quality controls to verify adequate processing of the target. The system also monitors the presence of inhibitors in the real-time PCR assay to signal a potentially false negative result.
- Xpert HPV Test results will be monitored during the course of every clinic and the Study Coordinator will be notified immediately if an “invalid / error / aborted” test result is obtained at any time. A relevant SOP will also be in place to support staff should such test results occur.

5.8 Collection of cost, health system implementation, and acceptability data

5.8.1. Economic evaluation data

A combination of health services and patient costs will be collected:

- Health services cost data will be collected using standardised templates from clinic staff and health service providers at district and provincial level; and from clinic records, study financial reports and service records. Costs include staff salaries, transport, logistics, consumables and equipment.
- Patient costs in money and time, including costs of care and transport of attending a well woman clinic, will be collected from a randomly selected sub-population of women attending their first clinic visit in each study phase using a study-specific electronic CRF (100 women in the pre-intervention phase; 100 women in the intervention phase at enrolment at 3-months follow-up).

Existing models will be calibrated using data available on HPV prevalence by age and type in PNG, and GLOBOCAN estimates on cervical cancer incidence and mortality.

5.8.2. Health system implementation data

Assessments use a mix of interview, observation, and records review to collect overlapping information that allows triangulation of findings.

- All assessments will be conducted in the pre-intervention phase, and at 6 months and 18 months after initiation of HPV Xpert screening. The focus of the pre-intervention assessment will be to document resources, turn-around-time and workflow in relation to existing VIA or Pap screening, for comparison purposes. Observational and interview tools used at 6 months and 18 months will include assessments of contextual changes, to help support any health service comparisons between existing screening and Xpert HPV screening.
- Quantitative data on health service delivery arrangements, staff work-flow, and patient-flow will be collected through structured observation and facility record reviews. Structured observation will use templates already tested in the PNG setting by our team; to assess staff work flow, patient-flow timing, service environments (including privacy) and client-provider interactions. Facility record reviews will use a pre-determined template to extract data on patient numbers, patient characteristics, and clinic staffing and resourcing. Data will be captured using an electronic tablet-based tool (e.g. RedCap) and exported to STATA (Stata 14, StataCorp LP,

TX, USA) for analysis.

- Quantitative and qualitative data will also be collected through focus group discussions (FGDs) and semi-structured interviews (SSIs) with members of the in-country clinical research team (3 focus groups, 10 interviews); health staff at provincial, district and clinic level (3 focus groups, 10 interviews); and national policy makers (4 interviews).

SSIs and FGDs will be documented in structured notetaking by interviewer and an observer. If feasible, some SSI data will be collected through electronic tablet-based tool. They will also be digitally recorded to enable cross-checking and additional analysis if needed. Qualitative data will be coded using qualitative data management software (e.g. NVivo, QSR, Ltd., Australia) and thematic analysis conducted to provide a ranked description of implementation issues from health staff and client perspectives.

5.8.3. Acceptability assessment

Acceptability will be evaluated from the perspective of women and health service providers:

- A study-specific electronic CRF will be administered by research staff to all participants at enrolment and clinical follow-up visits (as described above). This will measure satisfaction with services in all sites and evaluate acceptability and client opinions regarding the intervention. We will analyse quantitative data from acceptability CRFs and calculate the proportion of respondents who consider the intervention 'acceptable' or 'highly acceptable'. We will also review eligibility CRFs and study exclusion registers to calculate the number of women offered HPV point-of-care testing who declined to enrol in the study, and their reasons for not wishing to participate.
- To investigate women's preferences for service delivery and the wider societal and cultural contexts within which the acceptability of new interventions for cervical cancer are framed and perceived, we will conduct focus group discussions (FGDs) and semi-structured interviews (SSIs) with participants during the pre-intervention phase, and around the time of enrolment and at 3-month scheduled follow-up during the intervention phase (1-2 focus groups and 5-8 interviews per clinic at each time point). FGDs and SSIs will capture information on HPV and cervical cancer knowledge and attitudes, perceptions of clinical consultation, and service provision. This work will build on our earlier research in this same setting.[52]
- To investigate these issues and concepts from the perspective of health service providers, we will also conduct FGDs and SSIs among clinic staff, health professionals at provincial and national level, and members of our research team during the pre-intervention phase, and at 6 months and 18 months following the start of point-of-care HPV testing (1-2 focus groups and 5-8 interviews in each clinic setting at each time point; 1-2 interviews with national-level health staff at pre-intervention and 18 month time points).

Data collection will be aligned with the health systems implementation component where possible. Qualitative data from FGDs and SSIs will undergo thematic analysis and be used to draw conclusions about acceptability from client and health provider perspectives.

Table 6. Summary of health cost, health systems implementation and acceptability data collection activities

Activity	Purpose	Participants	Where conducted	When conducted	Conducted by
1. Collection of cost data	To estimate cost of implementing different cervical screening scenarios	All participating health facilities Sub-set of study participants	Participating clinics	Health facilities: pre-intervention phase, enrolment and 6 months after start of POC HPV testing Study participants: pre-intervention phase, enrolment and 3 months after start of POC HPV testing	IMR doctoral researcher in health economics / health systems
2. Observational study and facility records review	Collect data on changes to health service delivery arrangements, staff work-flow, and patient-flow.	All participating health facilities	Participating clinics	Pre-intervention phase, and at 6 months and 18 months after start of POC HPV testing	IMR doctoral researcher in health economics / health systems
3. Focus group discussions (FGDs) and semi-structured interviews (SSIs)	To assess feasibility, acceptability, health service preference, and sustainability of existing services and point-of-care HPV-DNA testing	In-country research team Health professionals at provincial and national level Sub-set of study participants	Participating clinics and other workplaces Participating clinics	Health staff and research team: pre-intervention phase, and at 6 months and 18 months after start of POC HPV testing Study participants: pre-intervention phase, enrolment and 3 months after start of POC HPV testing	IMR qualitative researcher
4. Acceptability CRFs	To estimate acceptability of the intervention among women attending for cervical screening	All study participants	Participating clinics	Pre-intervention phase, at enrolment and all follow up visits during the intervention phase	IMR clinical research staff

6 STATISTICAL CONSIDERATIONS

6.1 Sample size and potential power

Based on data from our recently completed Xpert HPV POC pilot study (N=1005),[43,44] and our earlier VIA study (N=614),[32] we estimate that around 14-18% of women in the target age group 30-59 years will have one or more hrHPV infection; that around 4-5% of women will have HPV16, 18 or 45; and that around 8-10% of women will have high-grade disease (HSIL or worse).[25]

The international literature indicates that hrHPV DNA testing alone has a sensitivity of around 90-94% and comparable specificity for the detection of CIN2 or more advanced disease.[30,34,54] In our recent pilot study, we found that Xpert HPV alone had around 92% sensitivity and 87% specificity for the detection of HSIL or more advanced disease, and that algorithms based on different test result scenarios (e.g. HPV-16+ alone; HPV-16/18/45+ alone) performed less well compared to the result of all hrHPV infections combined (Table 1, 2).[44]

Based on our earlier findings, we estimate that if 4000 women are screened in the current study, around 15% will be hrHPV positive (n=600) and around 8% (n=320) will have HSIL or more advanced disease.

In order to estimate the performance of different screening algorithms for the detection of HSIL or worse, we will also collect cervical specimens from a randomly selected 15% sub-population of women who are HPV negative (n=600).

A sample size of 4000 women screened would therefore provide primary and secondary outcome data on approximately 1200 women, and would be sufficient to:

- estimate performance characteristics (sensitivity, specificity, positive and negative predictive values) of different clinical algorithms with around **± 1.5% precision** (e.g. sensitivity of 93.0% would be estimated with 95% confidence interval (CI) of 91.5% - 94.5%);
- detect a **10% difference** in performance characteristics between alternative screening algorithms with α=0.05, β=0.20 (80% power).

6.2 Data analysis

Socio-demographic, behavioural and clinical information in study forms will be double data entered in-country, and following query resolution and other quality checks, data analysis will be carried out in STATA (Stata 14, StataCorp LP, TX, USA) by A/Prof Wand, the study statistician.

6.2.1. Performance of Xpert HPV test and alternative HPV-based screening algorithms for the early detection and treatment of cervical pre-cancer

We will calculate the sensitivity, specificity, positive and negative predictive values (with 95% confidence intervals) of the Xpert HPV Test to detect HSIL or greater, and calculate the proportion of all those with disease who receive ablative treatment at point-of-care.

The performance of Xpert HPV under different scenarios will also be calculated (e.g. any hrHPV+; HPV16+; HPV 16/18/45+; hrHPV+ at different PCR cycle thresholds and/or in combination with different biomarkers) in order to identify the combination of tests having the best performance characteristics.

We will compare the performance of alternative screening strategies using McNemar's test for sensitivity and specificity; and use a related statistical test for comparing predictive values.[55]

6.2.2. Cost-effectiveness

Standard cost-effectiveness analyses will be performed by identifying the cost-effectiveness frontier and calculating the incremental cost-effectiveness ratios (ICERs) of strategies on the frontier compared to the next most cost-effective strategy. We will calculate all cost-effectiveness ratios as cost per life year saved. A 3% discount rate will be used for both costs and benefits. The gross regional product (GRP) per capita for the relevant province will be used as the cost-effectiveness threshold for the evaluation. Formally, interventions with ICERs less than this value are considered "very cost-effective," according to WHO guidelines. Extensive sensitivity analysis of

the findings will be performed, using one-way and probabilistic sensitivity analysis approaches to assess the effect of variation in model assumptions on the health economic outcomes.

Prof Canfell and Dr Simms have developed a suite of models for assessing the effectiveness and cost-effectiveness of cervical screening that has been used to evaluate strategies in Australia, China, New Zealand and UK.[56,57] The platform has been developed over more than a decade through previous NHMRC-funded grants. In the current study, we will adapt this model to the PNG population using data on treatment costs and the prevalence of risk factors obtained in the current study and earlier research led by our group in this setting. We will configure the cervical screening, triage and treatment strategies, and populate the model with new cost and test positivity data from the current study.

6.2.3. Health system implementation

Health system implementation challenges and benefits will be evaluated in terms of patient-flow and turnaround-time; work-flow and staff time; training and supervision needs; procedures for finance, payment and health information; logistics and management; and shifts in client-provider relationships when moving from current routine screening (based on VIA alone or Pap test) to HPV-based screening.

6.2.4. Acceptability

Acceptability of the intervention from client and health provider perspectives will be analysed using quantitative and qualitative methods. Key findings from each approach will be triangulated in order to allow robust conclusions to be made regarding acceptability.

We will calculate the proportion of women who consider the intervention ‘acceptable’ or ‘highly acceptable’ using quantitative data collected in study-specific CRFs

Qualitative data will be collected from study participants, clinic staff, health managers and other key stakeholders in the pre-intervention and intervention phases of the study in focus group discussions (FGDs) and semi-structured interviews (SSIs) that will investigate women’s preferences for service delivery and the wider societal and cultural contexts within which the acceptability of new interventions for cervical cancer are framed and perceived.

6.2.5. Cervical cancer biomarkers

Laboratory performance characteristics (sensitivity, specificity, positive and negative predictive value) of self-collected compared with clinician-collected specimens for the detection of cervical cancer biomarkers will be calculated as proportions with 95% confidence intervals.

7 GOVERNANCE AND OVERSIGHT

7.1 Steering Committee

An in-country Steering Committee will be established to oversee the conduct of the study and to facilitate the translation of research findings into public health policy and clinical practice in PNG.

The Committee will meet prior to the start of the study and then approximately twice a year to review study progress.

Terms of Reference for the Steering Committee will be agreed at the first meeting and will be made available via the study website.

Minutes of Steering Committee meetings will be made available to study investigators and to in-country partners and stakeholders, including local-level provincial health committees and community liaison groups.

7.2 Study Operational Group (SOG)

The day-to-day management of the study will be overseen by this group, which will meet on a regular basis (every 2 weeks at the start of the study and then monthly) to review progress, including accrual and retention; eligibility and exclusions; diagnoses and treatment; data management issues; and logistical considerations.

The SOG will also ensure that the routine operations of the study proceed in accordance with the study protocol and study-specific standard operating procedures, and will engage with stakeholders and participating communities on an ongoing basis to provide progress updates on the conduct of the trial. SOG meetings will be coordinated by the Study Coordinator and chaired by the Chief Investigator (CI).

8 ETHICS / PROTECTION OF HUMAN SUBJECTS

The investigators will ensure that the study is conducted in accordance with the current revision of the Declaration of Helsinki, and with recommendations contained in the International Conference for Harmonisation Good Clinical Practice (ICH-GCP) guidelines.

8.1 Institutional Review Board (IRB)

The trial protocol will be submitted to the following Institutional Review Boards in Papua New Guinea and Australia:

- Papua New Guinea Institute of Medical Research (PNGIMR) Institutional Review Board (IRB)
- Medical Research Advisory Committee (MRAC) of the PNG National Department of Health
- Provincial Research Ethics Committees in participating provinces
- University of New South Wales (UNSW) Human Research Ethics Committee (HREC)

Study recruitment will not commence until written ethics approval has been obtained from all of the above committees.

Any amendments to the protocol or consent materials will be submitted for additional approval from the above before revised procedures or materials are placed into use.

In addition to the above, study investigators may seek ratification of the above approvals from their respective Institutional Review Boards.

8.2 Confidentiality

The confidentiality of participant information will be maintained at all times. Participant information will be identified by Study ID Number and/or Laboratory Sticker Number, and not by participant name. Biological samples will be tagged using the appropriate unique Laboratory Sticker Number.

The study ID Number and/or Laboratory Sticker Number will be a unique number that does not contain the patient initials.

Any notes with personal identifiers will only be accessible to clinical staff and other study personnel as authorised by the study PIs and specified in the Delegation Log.

No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the study Steering Committee.

8.3 Future use of stored specimens

Specimens collected in the trial will be stored for up to 10 years after completion of the last follow-up visit in order that they may be tested using new screening assays that may become available in future. As part of study informed consent procedures at enrolment, women joining the study will be asked to provide written consent for their specimens to be stored and potentially tested in future. In cases where such consent is not given, residual specimens will be destroyed immediately after all study laboratory testing has been conducted, and related quality control procedures completed. Specimens will be destroyed in accordance with study-specific operating procedures.

Additional tests to those described in the protocol will not be conducted on stored specimens without written permission from the above IRBs and HRECs.

8.4 Protocol deviations

The protocol will be adhered to for the entire duration of the study. In particular, no deviations from enrolment and exclusion criteria are permitted. Adherence to protocol will be monitored by the Study Coordinator and any suspected deviations reported immediately to the trial CI. The CI will report all confirmed protocol deviations to the IRBs / research ethics committees that approved the trial and if indicated seek amendments to the protocol.

9 DATA HANDLING AND RECORD KEEPING

9.1 Types of Data

The study will collect the following types of data:

- Socio-demographic data (e.g. marital status, educational attainment, employment)
- Care-seeking and household expenditure data (e.g. transport costs for clinical attendances)
- Behavioural data (e.g. sexual behaviour, smoking, betel nut consumption)
- Current and past clinical data (at enrolment, during follow-up)
- Treatment data (at enrolment, during follow-up)
- Routine antenatal laboratory data (haemoglobin, HIV, syphilis screening)
- HPV point-of-care and other laboratory test data
- Locator information
- Data derived from routine health services data on implementation costs associated with the intervention
- Data on the acceptability of the intervention from client and provider perspectives

9.2 Location and storage of source documents

Data collection tools (hard copy documents and electronic records) and supporting documentation will be kept securely as study source documents for potential review and/or audit during or after the trial. These will be stored at the PNGIMR in Goroka for 10 years after the end of the trial and will then be destroyed (hard copy documents by incineration; electronic records by file deletion / disk formatting).

- Socio-demographic, behavioural, clinical and laboratory information will be recorded in study-specific electronic Case Report Forms (eCRFs). This information will be identified by Study ID Number and/or Laboratory Sticker Number, and will not contain personal identifiers. Hard copies of these forms will be securely stored in a locked filing cabinet in each site at the end of every clinic day, and access to these records will be strictly controlled as indicated in the Delegation Log.
- Locator Forms, Informed Consent Forms and the Study Register containing personal identifiers (such as full name, home address, and village name) will be securely stored in a separate locked filing cabinet in accordance with ICH GCP Guidelines. This information is being collected in order to verify participants' identity and to facilitate community tracing and follow-up only, and will not be entered into study electronic databases.
- Electronic databases containing Xpert HPV test results will be stored on-site in the GeneXpert-associated laptop computer with daily on-site and weekly off-site backup.
- Qualitative information collected from participants will also be kept in a locked cabinet, and once entered onto the computer database will be password protected. Digitally-recorded interviews will likewise be stored in a password protected computer. None of the qualitative information will be stored with identifying details of participants.

9.3 Data collection and verification

The primary data will be collected by field staff at each participating site as specified in the study Delegation Log.

Electronic tablets running Mobile Data Studio (MDS) software (or similar electronic data capture software such as QDS, REDcap) will be used to administer interviews, enter extracted data from records review, and score checklists for audit and observation.

Electronic data capture has proven feasible in earlier field studies conducted by members of our collaborative group in this same setting (e.g. the Healthy Mother, Healthy Baby Study, led by Dr Morgan; the *Kauntim mi Tu* Integrated Bio-Behavioural Survey, led by Dr Kelly-Hanku). This approach will help improve inter-observer reliability while also eliminating the need for manual data

entry. Whilst the electronic tablets have been robust, paper versions of each electronic CRF (eCRF) will also be prepared and available for use should there be unforeseen problems arising with the use of electronic tablets.

For qualitative data capture, interviews will be digitally recorded and the research team will use complementary paper-based notebooks to allow capture of longer free-text responses.

All written data recorded in study registers and other documents will be legibly recorded in black or blue ink.

The Site Coordinator (or his/her designee) will be responsible for reviewing eCRFs, study logs and other documents during the course of each clinic day for completeness, validity and legibility, in accordance with study-specific operating procedures (SOPs):

- Clinic Attendance Tally Sheet
- Exclusions Register
- Study Register / Enrolment Log
- Informed Consent Forms
- Comprehension Checklists
- Locator Forms
- Enrolment Case Report Forms (CRFs)
- Follow-up CRFs
- Laboratory Test Results Log
- Cost of care-seeking, acceptability and socio-economic status CRF

Any possible errors identified by the Site Coordinator will be discussed with the staff member who completed the record/form and a final response recorded, in accordance with study-specific SOPs.

10 DISSEMINATION AND PUBLICATION OF STUDY FINDINGS

10.1 Study participants, communities and key stakeholders

The investigators are committed to maintaining on-going communication with participating sites and key stakeholders throughout the duration of the study. This will occur through the following mechanisms:

- Six-monthly newsletters prepared for study participants, local stakeholders and clinical staff at study sites documenting progress with each stage of the trial, emerging issues, upcoming events and new staffing. These newsletters will be disseminated to study sites and key local and national stakeholders including community leaders, national and provincial health departments, and other relevant organizations.
- Six-monthly summary activity reports for participating trial sites describing enrolment and retention figures, HPV tests conducted and the proportion positive.
- Six-monthly written updates to key stakeholders at national and provincial level providing an overview of the progress of the study. Presentations will also be provided to participating health authorities and stakeholder organizations as requested.
- A dedicated study-specific website will be established and used as a platform to disseminate progress updates. We will make these available throughout the course of the study as open access format reports and slide presentations; video updates from the field, including site visits and interviews with in-country policy makers; and interactive webinars (e.g. on study rationale, progress and anticipated impact delivered by senior scientists). The website will maintain an up to date database of research publications in the area of HPV-based cervical screening, and provide links to new findings as they become available in the scientific literature. We will evaluate the success and impact of these electronic resources according to the number of views received per day, number of resources downloaded and participation in webinars.
- A two-day National Policy Forum on completion of the study to enable senior health care managers, policy makers and development partners to engage with the research team in understanding the implications of our research findings for future public health policy. The forum will be preceded by early consultation with national and provincial policy-makers, to establish locally meaningful benchmarks for feasibility and cost-effectiveness, so that any impact statements presented in the forum can address both international norms and local priorities for sustainability.

10.2 Publication policy

The Investigator Group will develop a written Publication Policy that will be provided to the study Steering Committee for endorsement. Authorship will follow standard guidelines. Particular efforts will be made to ensure that PNG-based investigators have the opportunity to participate in authorship in a lead capacity.

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12 APPENDICES

Appendix		Version
1	Participant Information Sheet and Informed Consent Form (PISICF) - English	v0.1 (12Jul2017)
2	Participant Information Sheet and Informed Consent Form (PISICF) – tok pisin	v0.1 (12Jul2017)
3	Flipchart for community meetings and clinic-based information sessions	v0.1 (10Jul2017)
4	Comprehension Checklist	v0.1 (10Jul2017)
5	Literacy Check Form	v0.1 (10Jul2017)
6	Eligibility CRF	v0.1 (12Jul2017)
7	Guide for self-collection of vaginal specimens	v0.1(06Jun2017)
8	Enrolment CRF	v0.1 (12Jul2017)
9	3 month Follow-up CRF	v0.1 (12Jul2017)
10	12 month Follow-up CRF	v0.1 (12Jul2017)
11	Household expenditure and health costs CRF	v0.1(13Jul2017)
12	Locator Form	v0.1 (09Jul2017)
13	Participant Information Sheet and Informed Consent Form (PISICF) – individual or group interview (Participants) - English	v0.1 (13Jul2017)
14	Participant Information Sheet and Informed Consent Form (PISICF) – individual or group interview (Participants) – tok pisin	v0.1 (13Jul2017)
15	Semi-structured interview (SSI) and focus group discussion (FGD) Guide (Participants) Cost and Acceptability	v0.1 (13Jul2017)
16	Semi-structured interview (SSI) and focus group discussion (FGD) Guide (Health Workers)	v0.1 (13Jul2017)
17	Health Facility Observation, Records Review Guide	v0.1 (13Jul2017)