Papillomavirus Research 6 (2018) 70–76

Contents lists available at ScienceDirect

Papillomavirus Research

journal homepage: www.elsevier.com/locate/pvr

Performance of clinical screening algorithms comprising point-of-care HPV-DNA testing using self-collected vaginal specimens, and visual inspection of the cervix with acetic acid, for the detection of underlying high-grade squamous intraepithelial lesions in Papua New Guinea

Pamela J. Toliman[a,b], John M. Kaldor[b], Steven G. Badman[b], Josephine Gabuzzi[a], Selina Silim[a], Antonia Kumbia[c], Benny Kombuk[d], Zure Kombati[d], Gloria Munnull[a], Rebecca Guy[b], Lisa M. Vallely[b], Angela Kelly-Hanku[a], Handan Wand[b], Claire Ryan[e], Grace Tan[f], Julia Brotherton[f], Marion Saville[f], Glen D.L. Mola[g], Suzanne M. Garland[h,i], Sepehr N. Tabrizi[h,i], Andrew J. Vallely[a,*]

[a] Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea
[b] Kirby Institute, UNSW Sydney, Australia
[c] Goroka Provincial Hospital, Papua New Guinea
[d] Mt Hagen Provincial Hospital, Papua New Guinea
[e] Burnet Institute, Melbourne, Australia
[f] Victorian Cytology Service, Melbourne, Australia
[g] School of Medicine and Health Sciences, University of Papua New Guinea, Port Moresby, Papua New Guinea
[h] Department of Clinical Microbiology and Infectious Diseases, The Royal Women's Hospital, Parkville, Victoria, Australia
[i] Department of Obstetrics and Gynaecology, University of Melbourne, Victoria, Australia

ARTICLE INFO

Keywords:
Cervical cancer
HPV
Screening
Self-collect
Papua New Guinea
Visual inspection with acetic acid

ABSTRACT

The performance of different clinical screening algorithms comprising point-of-care HPV-DNA testing using self-collected vaginal ('V') specimens, and visual inspection of the cervix with acetic acid (VIA) was evaluated in Papua New Guinea.

Women aged 30–59 years provided V specimens that were tested at point-of-care using the Xpert HPV Test (Cepheid, Sunnyvale, CA). A clinician-collected cervical ('C') specimen was then collected for point-of-care Xpert testing, and liquid-based cytology (LBC). Following this, VIA examination was conducted, blind to HPV test results, and ablative cervical cryotherapy provided if indicated. Detection of high-grade squamous intraepithelial lesion (HSIL) by LBC was the reference standard used to evaluate clinical screening algorithms.

Of 1005 women, 36 had HSIL+. Xpert HPV Test performance using V specimens (sensitivity 91.7%, specificity 87.0%, PPV 34.0%, NPV 99.3%) was superior to VIA examination alone (51.5%, 81.4%, 17.5%, 95.6% respectively) in predicting underlying HSIL+. A screening algorithm comprising V specimen HPV testing followed by VIA examination had low sensitivity (45.5%) but comparable specificity, PPV and NPV to HPV testing alone (96.3%, 45.5%, 96.3% respectively).

List of abbreviations: DNA, Deoxyribonucleic Acid; HSIL, High-grade Squamous Intraepithelial Lesion; HPV, Human Papillomavirus; LMICs, Low- and Middle-Income Countries; PNG, Papua New Guinea; VIA, Visual Inspection with Acetic Acid; WHO, World Health Organization

* Correspondence to: Kirby Institute, UNSW Sydney, Wallace Wurth Building, High Street, Kensington, NSW 2052, Australia.
E-mail addresses: ptoliman@kirby.unsw.edu.au (P.J. Toliman), jkaldor@kirby.unsw.edu.au (J.M. Kaldor), sbadman@kirby.unsw.edu.au (S.G. Badman), josephine.gabuzzi@pngimr.org.pg (J. Gabuzzi), selina.silim@pngimr.org.pg (S. Silim), kumbiameri@yahoo.com.au (A. Kumbia), bennykombuk@yahoo.com (B. Kombuk), zure.kombati@whhs.gov.pg (Z. Kombati), gloria.munnull@pngimr.org.pg (G. Munnull), rguy@kirby.unsw.edu.au (R. Guy), lvallely@kirby.unsw.edu.au (L.M. Vallely), a.kelly@unsw.edu.au (A. Kelly-Hanku), hwand@kirby.unsw.edu.au (H. Wand), claire.nightingale14@gmail.com (C. Ryan), gtan@vcs.org.au (G. Tan), jbrother@vcs.org.au (J. Brotherton), msaville@vcs.org.au (M. Saville), glenmola@dg.com.pg (G.D.L. Mola), suzanne.garland@thewomens.org.au (S.M. Garland), sepehr.tabrizi@thewomens.org.au (S.N. Tabrizi), avallely@kirby.unsw.edu.au (A.J. Vallely).

https://doi.org/10.1016/j.pvr.2018.10.009
Received 13 August 2018; Received in revised form 16 October 2018; Accepted 29 October 2018
Available online 01 November 2018

A 'test-and-treat' screening algorithm based on point-of-care HPV testing of V specimens had superior performance compared with either VIA examination alone, or a combined screening algorithm comprising HPV testing plus VIA.

1. Background

Papua New Guinea (PNG) has among the highest estimated burdens of cervical cancer globally (estimated age-standardised incidence rate 34.5 per 100,000; age-standardised mortality rate 21.7/100,000) [1]. Cervical cancer is the leading cause of cancer mortality among women with an estimated 1000–1500 deaths every year [2,3]. The high burden of cervical cancer in PNG could be substantially alleviated through the implementation of an effective and accessible national cervical screening program, with associated medical referrals as needed [2,4–6].

A cervical screening initiative for women in PNG was established in 1999 by a non-governmental Australian-supported charity (the MeriPath program), and provided a service from more than 30 health facilities in 16 provinces [7]. The program was able to achieve only modest coverage however, with around 45,000 women screened over ten years (2001–2011), representing less than 4% of the target population aged 20–59 years. Also, as specimens were sent to Australia for testing, more than half of those found to have high-grade disease were lost to follow-up, due to the delay between testing and recall. Recognizing these constraints, a *Ministerial Task Force on Cervical Cancer* called for locally-appropriate models of cervical screening and early treatment to be evaluated in PNG [8]. The Task Force favoured the 'screen and treat' approach endorsed by the World Health Organization (WHO) for use in low- and middle-income countries (LMICs), based on visualization of the cervix after acetic acid (VIA), followed by ablative cervical cryotherapy [8]. Many countries have however experienced difficulties scaling up VIA, while maintaining adequate quality, and have reported much lower sensitivity and specificity than had been achieved in research settings [9–11]. In PNG, the first evaluation of VIA plus cryotherapy for primary cervical screening was recently completed (n = 614) and found poor correlation between VIA and laboratory-based high-risk HPV test results [12]. 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, to enable immediate clinical decision making.

The recognition that persistent infection with high-risk types of HPV (hrHPV) is the necessary factor for development of both cervical pre-cancer and cancer [13] has led to the development of new screening technologies that detect HPV-DNA directly. In the last decade, the potential health impact of HPV-DNA based testing as the primary screening pathway has been demonstrated in large-scale randomised trials and prospective studies [14–16]. These findings led to recommendations in Europe, the United States, Australia and other high-income settings for cervical screening programs to adopt HPV-DNA testing [16–19] for primary cervical screening; and to the WHO recommendation that HPV-DNA testing be integrated into screening programs in LMICs [20]. A key issue for LMICs is the cost and technical expertise required for HPV testing. In addition, the requirement that clinicians collect specimens stands as a substantial barrier, as it has with cytology screening, because of the requirement for a pelvic examination. Therefore, the development of an accurate, easy-to-use, nucleic acid amplification test (NAAT) that provides laboratory quality results within 60 min has been a significant advance (GeneXpert, Cepheid, Sunnyvale, CA). The Xpert HPV Test detects 14 oncogenic HPV types, including identifying HPV16, 18 and 45, and its performance for the detection of histologically-confirmed cervical pre-cancer found to be comparable to laboratory-based HPV molecular assays [18,21,22]. The portable Xpert HPV Test can be conducted on an individual basis, and with suitable training and support, provided at point-of-care within routine clinical settings in LMICs [23,24]. Furthermore, we recently demonstrated the excellent performance of self-collected vaginal specimens compared with clinician-collected cervical specimens tested on Xpert HPV [23], and compared with laboratory-based Roche Cobas 4800 HPV and Hologic Aptima HPV assays [25]. The use of self-collected vaginal specimens for hrHPV testing offers a cervical screening solution that is rapid and minimally-invasive for women, and not dependent on advanced laboratory or clinical expertise.

The WHO has recommended sequential 'test and treat' algorithms be evaluated in LMICs in order to inform future policy and practice [20]. Two recent studies evaluated a two-stage screening strategy comprising laboratory-based hrHPV testing and VIA examination [26,27], but neither provided same-day 'screen and treat' services as advocated by WHO. A third study recently sought to evaluate Xpert HPV testing at point-of-care followed by VIA, but failed to ascertain disease status among women who tested HPV negative and was therefore unable to evaluate the performance of the combination algorithm to detect high-grade disease [24].

The objective of the current study was to evaluate the performance of different clinical screening algorithms comprising point-of-care Xpert HPV testing and VIA examination for the detection of high-grade cervical disease. This study was to determine which clinical screening algorithm would be most suitable for further evaluation in large-scale screening studies to inform future population-based cervical screening in PNG and other high-burden, low-resource settings.

2. Materials and methods

2.1. Setting

The study was conducted in two well-women clinics at Goroka General Hospital (Goroka, Eastern Highlands Province) and Mt Hagen General Hospital (Mt Hagen, Western Highlands Province), PNG, which serve provincial populations of 579,825 and 362,850 respectively [28]. Both clinics provide cervical 'screen and treat' services based on VIA examination alone followed by ablative cervical cryotherapy if indicated. Clinic staff at both sites attended intensive residential training courses in VIA examination and cryotherapy, at an internationally-recognised training centre in Thailand. This was followed by on-site assessment and staff accreditation in PNG, within six-months of initial training. Day-to-day support supervision of clinical staff conducting VIA was provided by a consultant gynaecologist at each site (AK, BK).

2.2. Study design and participants

Cross-sectional study among 1000 women attending cervical screening services in PNG. Sample size requirements were based on estimated prevalences of hrHPV infection and ASC-H / HSIL of around 15–20% and 4–7% respectively in this population [2,12]. A sample size of 1000 women would enable test performance characteristics to be estimated with approximately 3–6% precision.

Clinic attendees aged 30–59 years (the target age for VIA-based screening in PNG) with an intact cervix were invited by clinic staff to participate in the study and, following completion of informed consent procedures, were enrolled sequentially.

2.3. Study procedures

A short face-to-face interview was conducted by a member of the clinical research team to collect socio-demographic, behavioural and clinical information. Clinic staff used a pictorial guide to advise women

how to collect a mid-cavity vaginal specimen using a cytobrush ("Just for Me", Preventative Oncology International, Cleveland Heights, Ohio). Each self-collected vaginal (V) specimen was provided immediately after collection to an onsite laboratory technician, who placed it into 20 ml ThinPrep PreservCyt (Hologic, Marlborough, MA) solution. A 1 mL sample of PreservCyt fluid was then tested using the Xpert HPV test as per manufacturer's instructions. Following self-collection, women underwent a clinical examination conducted by a research nurse or health extension officer during which a clinician-collected cervical (C) specimen was obtained using the same type of cytobrush. Specimen processing and testing for hrHPV was identical to that described above. VIA examination was then carried out by the same clinician who was blinded to hrHPV test results. Women with a positive VIA examination (acetowhite staining observed within the squamo-columnar junction) were offered same-day ablative cryotherapy. Women with extensive acetowhite staining, those with cervical lesions considered suspicious of possible cancer, and cases where it was not possible to visualise the entire squamo-columnar junction, were referred to the Gynaecology Department at participating provincial hospitals, as per study-specific standard operating procedures.

Cervical PreservCyt specimens were stored at 4 °C for a median of 13 months (interquartile range 11–18 months) before cool shipment to Melbourne, Australia, where additional molecular testing (data not presented) was conducted at the Department of Clinical Microbiology and Infectious Diseases, The Royal Women's Hospital. Following this, liquid based cytology (LBC) was performed on residual PreservCyt specimens at the Victorian Cytology Service (VCS), a National Association of Testing Authorities (NATA) / Royal College of Pathologists Australia (RCPA) accredited laboratory. All cervical slides were assessed by experienced assessors and any slides with High-Grade Squamous Intraepithelial Lesion (HSIL) were confirmed by a senior cytopathologist [29]. In our analysis, high-grade disease included all those with the cytological finding of HSIL or worse. Women who were not offered ablative cryotherapy at point-of-care but who were subsequently found to have HSIL were traced in the community and advised to return for further treatment.

2.4. Statistical analysis

All test result data were entered into an MS Excel study-specific database and independently checked against source data (e.g. paper-based case record forms; electronic GeneXpert.GXX files) at designated time points during the study, following completion of fieldwork, and on completion of offsite laboratory testing.

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the following algorithms for the detection of underlying HSIL were then calculated: VIA examination alone; Xpert HPV Test alone; and different possible combinations of VIA examination and Xpert HPV Test results (e.g. positive for HPV16 *and* VIA positive vs. hrHPV negative). A specific objective was to evaluate the possible benefit of VIA examination among women who test positive for hrHPV infection (e.g. detection of underlying HSIL among women who test positive for any hrHPV *and* are VIA positive, compared with detection among women who are either (a) hrHPV negative OR (b) positive for any hrHPV infection but subsequently have a negative VIA examination).

Performance measures were calculated as proportions with 95% confidence intervals (CIs) for the detection of high-grade disease, based on a disease threshold of HSIL [29]. Chi squared or Fisher's exact tests (as appropriate) and the Wilcoxon rank-sum tests were used to compare the outcomes of interest between groups (e.g. differences in socio-demographic and behavioural characteristics by hrHPV status). The p values < 0.05 were considered as statistically significant. All statistical analyses were performed using Stata version 14.1 (StataCorp, College Station, TX).

Table 1
Selected socio-demographic, behavioural and clinical characteristics of study participants by hrHPV status of self-collected vaginal specimen (N = 1005).

	hrHPV Positive n = 167 (16.6%)	hrHPV Negative n = 838 (83.3%)	p value*
Age[a], median (IQR) (years)	38 (IQR 34–43)	39 (IQR 35–45)	–
Marital status[b]			
- Married	146	750	0.473
- Single, Divorced or Widowed	20	85	
Education[c] (highest level)			
- Never attended school	55 (33.1)	289 (34.9)	0.113
- Primary school	66 (39.8)	381 (47.1)	
- Secondary school	23 (13.9)	92 (11.1)	
- Tertiary school	22 (13.3)	65 (7.9)	
Age of sexual debut[d]:			
- Median years (IQR)	18 (IQR 17–20)	18 (IQR 16–20)	0.215**
Lifetime number of sexual partners[e]			
- Median (IQR)	1 (IQR 1–2)	1 (IQR 1–2)	–
Number of partners in last month[f]			
- Median (IQR)	1 (IQR 1–1)	1 (IQR 1–1)	0.328**
Previous cervical screening[d]	29 (17.4)	150 (17.9)	0.802
HPV vaccination[d]	0 (0)	2 (0.2)	0.665***
Previous STI/genital infection[g]	78 (47.0)	373 (44.6)	0.631
Current genital symptoms[d]	112 (67.1)	501 (59.9)	0.084
- Genital warts[h]	4 (2.4)	5 (0.6)	0.076***
- Genital discharge[i]	115 (71.0)	570 (68.8)	0.186
VIA positive[j]	51 (34.5)	95 (12.7)	< 0.001
High-grade disease (HSIL or worse)[k]	33 (6.3)	3 (0.70)	< 0.001

[a] n = 1004.
[b] n = 1001.
[c] n = 993.
[d] n = 1003.
[e] n = 994.
[f] n = 968.
[g] n = 1002.
[h] n = 988.
[i] n = 990.
[j] n = 895.
[k] n = 527.
* Chi-square unless otherwise indicated.
** Wilcoxon rank sum test.
*** Fisher's exact test.

3. Results

3.1. Sociodemographic, behavioural and clinical characteristics

A total of 1005 women were recruited (614 and 391 at sites 1 and 2, respectively) between November 2014 and October 2015. More than 90% of those invited to participate in the study were enrolled. All offsite laboratory investigations were completed by October 2016. Overall, the prevalence of hrHPV infection (based on testing self-collected vaginal specimens) was 16.6% (167/1005; Table 1). Women with hrHPV were marginally younger than hrHPV negative women (median age 38 years vs. 39 years) and significantly more likely to have a positive VIA examination (34.5% vs. 12.7%; $p < 0.001$). No statistically significant differences were observed in marital status, education level, age of sexual debut, and other behavioural and clinical characteristics between hrHPV positive and negative women (Table 1). A majority of participants had not previously undergone cervical screening (n = 824, 82.2%), and only two (0.2%) reported having received HPV vaccine. Approximately two-thirds (n = 685, 69.2%) of women reported vaginal discharge at the time of their clinic visit. VIA examination was conducted on 895 women (89.1%), of whom 16.3% (146/895) were VIA

positive (Table 1). It was not possible to complete VIA examination in 110 women (10.9%) due to an inability to visualise the entire squamo-columnar junction and/or concomitant cervicitis.

3.1.1. Cytology findings

A total of 991 cervical specimens were available for liquid based cytology of which 527 (53.2%) were considered to be satisfactory for evaluation by experienced assessors and cytopathologists at VCS. High-grade disease (HSIL or worse) was detected in 6.8% of specimens (36/527), the majority among hrHPV positive women (91.7%; 33/36) (Table 2). Evidence of cervical cancer was detected in nine (1.7%) women, all of whom were hrHPV positive on their self-collected vaginal specimen.

Of the 464 unsatisfactory specimens, 14 (3.0%) could not be processed because of low sample volume, and 450 (97.0%) did not contain sufficient cellular material to be deemed satisfactory for evaluation. A liquid based specimen is deemed to be unsatisfactory when there are fewer than 5000 visible squamous cells on the slide [29]. The processing of specimens for laboratory-based molecular assays prior to conducting LBC was considered a key factor. There was no difference in the proportion of satisfactory and unsatisfactory LBC specimens from hrHPV positive women (18.4% vs. 14.9% respectively; $p = 0.137$). The quality of C specimens varied by site: 46.1% (281/610) of specimens collected in site 1 were satisfactory compared with 64.6% (246/381) in site 2 ($p < 0.001$). There was no significant difference in overall hrHPV prevalence between site 1 and site 2 (16.6% vs. 16.7%; $p = 0.996$); and no difference between sites in the proportion of satisfactory LBC specimens from hrHPV positive women (18.9% vs. 17.9%; $p = 0.773$).

3.1.2. Performance of different clinical algorithms for the detection of high-grade disease (HSIL or worse)

VIA examination was completed in 96.7% (33/36) of women identified with high-grade disease, while for three women it was not possible to complete the examination because the squamo-columnar junction was not fully visualised. Overall, 48.5% (16/33) of women with high-grade disease had negative VIA examinations. Of the 17 women with high-grade disease who were VIA positive, 11 (64.7%) received same-day cryotherapy; three (17.6%) were referred for specialist review due to suspected cervical cancer; one (5.9%) was pregnant (cryotherapy was deferred); and two (11.8%) were Xpert HPV negative on both their self-collected vaginal and clinician-collected cervical specimens and did not receive cryotherapy, but were later contacted and asked to return for review.

VIA examination alone had low sensitivity (51.5%; 95%CI: 33.5–69.2) and PPV (17.5%; 95%CI: 10.6–26.6), and moderate-high specificity (81.4%; 95%CI: 77.3–84.9) and NPV (95.6; 95%CI: 93.0–97.5), for the detection of cytological high-grade disease (Table 3). VIA alone had comparable sensitivity at each site (55.6% and 46.7%; $p = 0.20$) but greater specificity in site 1 compared to site 2 (96.0 vs. 68.8; $p < 0.05$) (data not presented).

Point-of-care Xpert HPV testing of self-collected vaginal specimens had high sensitivity (91.7%; 95%CI: 77.5–98.2), specificity (87.0%; 95%CI: 83.7–89.8), PPV (34.0%; 95%CI: 24.7–44.3), and NPV (99.3; 95%CI: 98.0–99.9) for the detection of high-grade disease based on Xpert test results for all HPV types combined ('Any hrHPV'; Table 3). The performance of self-collected vaginal and clinician collected cervical specimens for the detection of high-grade disease were comparable using the GeneXpert platform (Table 3). Clinical algorithms based on HPV16 alone, HPV18/45 alone, or HPV16/18/45 alone, had lower sensitivity (66.7–86.4%) but greater specificity (96.8–99.1%) than an algorithm based on detection of any hrHPV type (sensitivity 91.7%, specificity 87.0%) (data not presented). The performance of HPV-based clinical algorithms for the detection of underlying HSIL did not vary significantly by clinical site (data not presented).

Xpert HPV Test followed by VIA examination had marginally higher specificity (96.3% vs. 87.0%) but significantly lower sensitivity (45.5% vs. 91.7%; $p < 0.001$) than Xpert HPV Test alone (Table 3). The addition of VIA to algorithms based on HPV16 alone, HPV18/45 alone, or HPV16/18/45 alone, also led to substantial and statistically significant reductions in sensitivity (data not presented).

4. Discussion

This is the first study to evaluate a 'test and treat' cervical screening algorithm comprising point-of-care Xpert HPV testing using self-collected vaginal specimens followed by VIA examination; a sequential algorithm that WHO has recommended be evaluated as a priority [19]. We found that the combination algorithm had significantly lower performance for the detection of high-grade cervical disease compared to point-of-care hrHPV testing alone in this setting. A 'test and treat' algorithm based on Xpert HPV alone would have appropriately treated 92% (33/36) of all women with high-grade disease (HSIL or worse); over-treated 13% (64/491) of women without disease; and would not have detected and treated 8% (3/36) of women with high-grade disease. A 'screen and treat' algorithm using VIA alone would have appropriately treated 51.5% (17/33) of women with HSIL or worse; over-treated 18.6% (80/429) of women without disease; and missed 48.5% (16/33) of women with underlying high-grade disease. The clinical performance of a combination algorithm (HPV testing followed by VIA) was comparable to that of VIA alone (45.5% appropriately treated; 3.7% over-treated; 54.5% missed). We further found that clinical algorithms based on partial genotyping (e.g. providing same-day treatment to women who were HPV 16 or HPV 16/18/45 positive, and deferring treatment of those with other hrHPV infections), had sub-optimal performance compared to providing same-day treatment to all hrHPV positive women, irrespective of hrHPV type, in this setting. Incorporating partial genotyping into combination algorithms comprising HPV testing and VIA did not improve their performance characteristics. Overall, our findings suggest that a 'test and treat' strategy comprising point-of-care Xpert HPV testing of self-collected vaginal specimens followed by same-day cervical ablation (e.g. using cryotherapy or

Table 2
Cytology findings by HPV status[a] (n = 527).

Cytology	hrHPV Negative	Any hrHPV Positive	HPV 16 Positive	HPV18/45 Positive	Other hrHPV Positive
Negative (n = 433)	394 (91.6)	39 (9.0)	5 (1.2)	2 (0.5)	34 (7.9)
LSIL (n = 31)	20 (64.5)	11 (35.5)	1 (3.2)	1 (3.2)	10 (32.3)
AGUS[b] (n = 4)	3 (0.7)	1 (0.3)	1 (0.3)	0 (0)	0 (0)
ASC-H[c] (n = 23)	10 (43.5)	13 (56.5)	4 (17.4)	1 (4.3)	10 (43.5)
HSIL (n = 27)	3 (11.1)	24 (88.9)	9 (33.3)	6 (22.2)	17 (63.0)
Cancer (n = 9)	0 (0)	9 (100)	5 (55.6)	0	5 (55.6)

[a] Based on self-collected vaginal specimen tested on Xpert HPV and includes infection with multiple hrHPV types.
[b] Atypical Glandular Cells of Undetermined Significance.
[c] Atypical Squamous Cells – cannot exclude HSIL.

Table 3
Performance of VIA examination and HPV Xpert test algorithms for detection of high-grade disease.

Screening Algorithm			High-Grade Disease		Percentage %			
			Positive n (%)	Negative n (%)	Sensitivity [95% CI]	Specificity [95% CI]	PPV [95% CI]	NPV [95% CI]
Algorithm 1	**POSITIVE**	**VIA +**	17 (3.7)	80 (17.3)	**51.5**	**81.4**	**17.5**	**95.6**
(n = 462)	**NEGATIVE**	**VIA -**	16 (3.5)	349 (75.5)	[33.5–69.2]	[77.3–84.9]	[10.6–26.6]	[93.0–97.5]
Algorithm 2	**POSITIVE**	**Any hrHPV +** (hrHPV test on self-collected specimen)	33 (6.3)	64 (12.1)	**91.7** [77.5–98.2]	**87.0** [83.7–89.8]	**34.0** [24.7–44.3]	**99.3** [98.0–99.9]
(n = 527)	**NEGATIVE**	**Any hrHPV –** (hrHPV test on self-collected specimen)	3 (0.6)	427 (81.0)				
Algorithm 3	**POSITIVE**	**Any hrHPV +** (hrHPV test on clinician-collected specimen)	33 (6.2)	48 (9.1)	**91.7** [77.5–98.2]	**90.3** [87.3–92.7]	**40.7** [29.9–52.2]	**99.3** [98.1–99.9]
(n = 529)	**NEGATIVE**	**Any hrHPV –** (hrHPV test on clinician-collected specimen)	3 (0.6)	445 (84.1)				
Algorithm 4	**POSITIVE**	**Any hrHPV** + and **VIA +** (hrHPV test on self-collected specimen)	15 (2.9)	18 (3.5)	**45.5** [28.1–63.6]	**96.3** [94.2–97.8]	**45.5** [28.1–63.6]	**96.3** [94.2–97.8]
(n = 515)	**NEGATIVE**	**Any hrHPV –** or **Any hrHPV** + and **VIA -** (hrHPV test on self-collected specimen)	18 (3.5)	464 (90.1)				

Algorithm 1 versus Algorithm 2; sensitivity $p < 0.01$, specificity $p = 0.02$, PPV $p < 0.01$, NPV $p < 0.01$.
Algorithm 1 versus algorithm 3; sensitivity $p < 0.01$, specificity $p < 0.01$, PPV $p < 0.01$, NPV $p < 0.01$.
Algorithm 1 versus algorithm 4; sensitivity $p = 0.62$, specificity $p < 0.01$, PPV $p < 0.01$, NPV $p = 0.63$.
Algorithm 2 versus algorithm 4; sensitivity $p < 0.01$, specificity $p < 0.01$, PPV $p = 0.24$, NPV $p < 0.01$.

newly-available portable thermocoagulation devices) without VIA-based triage would have excellent performance for the detection and treatment of high-grade disease, and be feasible to implement in the low-resource setting of PNG.

We used liquid-based cytology as our diagnostic reference standard (with HSIL the pre-defined disease threshold), rather than histology (with a disease threshold of CIN2), which is often the preferred diagnostic reference (or 'gold standard'), because it was not feasible to provide colposcopy, or to collect four-quadrant cervical biopsies for histological examination, due to shortages of experienced clinical staff and logistical considerations in this setting. Colposcopy and biopsy are also not considered feasible or sustainable within existing health services for population-level screening in PNG. Researchers in other low-resource settings have also used HSIL as their preferred disease threshold for similar reasons [26,30,31]. In the Australian setting, around 80% of HSIL diagnoses at VCS are confirmed histologically as high-grade disease (cervical intraepithelial neoplasia grade 2 or worse), with the majority (around 54%) diagnosed as CIN3 or worse [32]. The performance of cytology is greatly dependent on specimen collection and storage, both of which are challenging in settings such as PNG, so that although our specimens were assessed at VCS, it is possible that the level of correlation between HSIL diagnoses and CIN2+ may have been less than 80% in our study. An advantage of our chosen reference standard was that it allowed us to collect specimens and to measure our primary study outcome among women who were hrHPV negative, among whom it would not have been ethical to take four-quadrant cervical biopsies for histological examination, had we selected the histological endpoint of CIN2 as our disease threshold.

The performance characteristics of our 'test and treat' algorithm based on point-of-care Xpert HPV testing alone might be considered sub-optimal compared with current practice in many high-income countries (hrHPV testing followed by liquid based cytology if hrHPV-positive, with referral for colposcopy and biopsy if HSIL or worse) [33–38]. However, same-day 'test and treat' strategies offer considerable advantages over two- or three-step algorithms in low-resource settings that offset potential differences in algorithm performance. Loss to follow-up among women identified as having cervical disease at initial screening is a major concern in many low-resource settings [39,40] and was substantial in an earlier Pap test based program in PNG [7]. This risk is substantially reduced in screening programs based on hrHPV 'test and treat', because the majority of women can be offered same-day treatment. In the current study, we demonstrated the operational feasibility of providing point-of-care Xpert HPV testing and ablative cervical cryotherapy in routine clinical settings in PNG. Compared to screening algorithms that include cytology and colposcopically directed biopsy, our 'test and treat' approach appears readily scalable and would require substantially fewer resources (including senior clinical staff training, capital costs, logistical support, technical oversight). Overall, these advantages suggest that HPV 'test and treat' strategies are likely to have a far greater impact at population level than more complex algorithms in low-resource settings, despite possible differences in performance characteristics.

In addition, the specificity and predictive value of future HPV-based point-of-care strategies could be further enhanced by the inclusion of highly-specific assays into clinical screening algorithms, such as those that target HPV oncoproteins E6 and E7, some of which have potential to be provided at point-of-care [41,42]. Such combination algorithms are likely to be required in future as successful screening programs are scaled up, and the overall burden of disease falls among women in such settings, in order to maintain optimal predictive values. It would be anticipated that the underlying prevalence of HSIL will be lower in second and subsequent rounds of screening, perhaps requiring an algorithm or test with better specificity than is needed in first (prevalence) round screening.

To date three other studies have assessed screening algorithms comprising VIA examination and hrHPV testing [9,24,27]. Two of these studies conducted off-site laboratory hrHPV testing and thus were unable to provide a same-day screen and treat service [9,27]. Initial treatment decisions were therefore made on the basis of VIA examination findings alone, which had low sensitivity and predictive value. In India, the sensitivity and specificity of hrHPV testing alone for the detection of histologically-proven cervical intraepithelial neoplasia

(CIN) grade 2 or above was 73.4% and 95.7%; and for a combination algorithm comprising VIA followed by hrHPV testing, was 45.0% and 99.6%, respectively [9]. HPV testing alone had 93.8% sensitivity and 95.6% specificity for the detection of CIN3 or worse; and an algorithm comprising VIA and hrHPV testing had substantially lower sensitivity (64.2% vs. 93.8%), but comparable specificity [9]. Women found to be hrHPV positive were recalled for repeat clinical review and treatment, but substantial losses to follow-up were observed [9]. Similar findings on the performance of hrHPV testing alone, and in combination with VIA, for the detection of CIN2 or worse were reported in Cameroon [27]. The most recent study assessed an algorithm comprising Xpert HPV testing at point-of-care followed by VIA, but failed to ascertain disease status among women who tested hrHPV negative, and was therefore unable to fully evaluate the performance of the combination algorithm to detect high-grade disease [24]. In contrast to these earlier studies, all women in our study, irrespective of hrHPV test result, were offered VIA examination and asked to provide a specimen for liquid-based cytology in order to detect underlying HSIL.

Our study had a number of potential limitations. The modest sample size, although sufficient to estimate performance measures with a high degree of precision, nonetheless meant that the total number of HSIL cases was comparatively small (n = 36). A high proportion (46%) of cervical specimens were considered unsatisfactory for LBC due to poor sample quality, and were excluded from analysis. While there was a lengthy storage period between collection and LBC analysis (median 13 months), low volume and poor cellularity were the primary reasons for samples being deemed unsatisfactory and not the degradation of nuclear quality, which is a documented effect of long-term storage [43]. A key contributing factor to C specimen depletion was the fact LBC was performed last in a series of laboratory tests (data not reported). Although the quality of clinician-collected cervical specimens for LBC analysis varied by site, we did not see any difference in hrHPV infection by site or by sample quality. Overall, this reduced the precision of estimated performance characteristics but we do not believe that this introduced bias, and an analysis of the performance of the algorithm based on hrHPV testing alone by clinical site found no significant differences. Improved staff training, support supervision and clinical mentoring are currently being implemented to improve the quality of specimen collection in future projects.

Despite considerable investment in overseas and local training for staff, variability in the performance of VIA was observed across the sites. This highlights the challenge in maintaining adequate quality for a test contingent on, among other things, an unobstructed view of the SCJ and the appearance of the cervix that is uncomplicated by other genital infections. The observed poor performance of an algorithm based solely on VIA as well as that hrHPV testing and VIA further add to a growing body of evidence suggesting that VIA is not sufficiently accurate for clinical triage among hrHPV positive women, because it cannot readily discriminate between those with and without disease.

5. Conclusions

We have demonstrated the excellent performance characteristics of Xpert HPV for the detection of high-grade cervical disease using self-collected vaginal specimens tested at point-of-care, and found that performance was comparable to clinician-collected cervical specimens. We further demonstrated the feasibility of implementing an HPV-based point-of-care 'test and treat' screening strategy in routine clinical settings. These findings suggest that self-collected vaginal specimens could be used as the primary screening method in PNG and other high-burden, low-resource settings. This strategy has the potential to substantially reduce clinic waiting times and the need for all women to undergo a potentially uncomfortable and/or embarrassing pelvic examination (given that the majority of women attending for screening would be HPV negative), and would allow highly-skilled clinical staff to spend more time conducting clinical assessments and providing treatment to women at greatest risk of disease i.e. those having a positive Xpert HPV Test. The effectiveness, health system implementation requirements, acceptability and cost-effectiveness of this strategy warrant further evaluation in large-scale field trials.

CRediT authorship contribution statement

Pamela J. Toliman: Conceptualization, Data curation, Formal analysis, Investigation, Project administration, Writing – original draft. **John M. Kaldor:** Conceptualization, Funding acquisition, Writing – review & editing. **Steven G. Badman:** Investigation, Supervision. **Josephine Gabuzzi:** Data curation, Investigation. **Selina Silim:** Data curation, Investigation. **Antonia Kumbia:** Supervision. **Benny Kombuk:** Supervision. **Zure Kombati:** Supervision. **Gloria Munnull:** Data curation, Investigation. **Rebecca Guy:** Conceptualization. **Lisa M. Vallely:** Supervision. **Angela Kelly-Hanku:** Supervision. **Handan Wand:** Conceptualization, Formal analysis. **Claire Ryan:** Conceptualization, Supervision. **Grace Tan:** Investigation. **Julia Brotherton:** Writing – review & editing. **Marion Saville:** Writing – review & editing. **Glen D.L. Mola:** Conceptualization. **Suzanne M. Garland:** Conceptualization. **Sepehr N. Tabrizi:** Conceptualization, Funding acquisition. **Andrew J. Vallely:** Conceptualization, Funding acquisition, Resources, Writing – review & editing.

Acknowledgements

We sincerely thank the women who participated 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 donating Xpert HPV Test cartridges for this project. We acknowledge the generous core funding support provided to the PNGIMR by the Australian Aid Program, Department of Foreign Affairs and Trade, Government of Australia. JK is supported by an NHMRC Research Fellowship; AV is supported by an NHMRC Career Development Fellowship.

Ethics approval and consent to participate

Ethics approval was obtained from the Papua New Guinea Medical Research Advisory Committee (MRAC 14.28), the Institutional Review Board of the Papua New Guinea Institute of Medical Research (IRB 1306), and the Human Research Ethics Committee, UNSW Sydney (HC13268). All participants provided written informed consent prior to participation in the study by signature or witnessed thumbprint.

Consent for publication

All participants consented to the publication of information related to their participation in the study.

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was funded through the National Health and Medical Research Council of Australia (NHMRC) (1071269) and the Papua New

Guinea Institute of Medical Research (PNGIMR) Internal Competitive Research Award Scheme (297/1). The funders had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication.

References

[1] L.A. Torre, F. Bray, R.L. Siegel, J. Ferlay, J. Lortet-Tieulent, A. Jemal, Global cancer statistics, 2012, CA: Cancer J. Clin. 65 (2) (2015) 87–108.
[2] A. Vallely, G.D. Mola, J.M. Kaldor, Achieving control of cervical cancer in Papua New Guinea: what are the research and program priorities? PNG Med. J. 54 (3–4) (2011) 83–90.
[3] World Health Organization, Papua New Guinea - Cancer Country Profile Genever, World Health Organization Press, 2014.
[4] S.M. Garland, J.M. Brotherton, S.R. Skinner, M. Pitts, M. Saville, G. Mola, et al., Human papillomavirus and cervical cancer in Australasia and Oceania: risk-factors, epidemiology and prevention, Vaccine 26 (Suppl 12) (2008) M80–M88.
[5] J. Goyen, Cervical screening in Papua New Guinea, Cytoletter 36 (1) (2012) 20–23.
[6] J. Obel, J. McKenzie, L.E. Buenconsejo-Lum, A.M. Durand, A. Ekeroma, Y. Souares, et al., Mapping HPV vaccination and cervical cancer screening practice in the pacific region-strengthening national and regional cervical cancer prevention, Asian Pac. J. Cancer Prev. 16 (8) (2015) 3435–3442.
[7] J. Goyen, Cervical screening in Papua New Guinea: 10 years experience of the MeriPath program. Proceedings of the 47th Annual Symposium of the Medical Society of Papua New Guinea; Kimbe, West New Britain, 2011.
[8] G. Mola, E. Posanai, L. Augerea, W. Lagani, A.B. Amoa, M. O'Connor, et al., Final Report of the Ministerial Task Force on Prevention and Treatment of Cancer of the Cervix in PNG, Government of Papua New Guinea, Port Moresby, 2009.
[9] P. Basu, S. Mittal, D. Banerjee, P. Singh, C. Panda, S. Dutta, et al., Diagnostic accuracy of VIA and HPV detection as primary and sequential screening tests in a cervical cancer screening demonstration project in India, Int. J. Cancer 137 (4) (2015) 859–867.
[10] J. Cuzick, M. Arbyn, R. Sankaranarayanan, V. Tsu, G. Ronco, M.H. Mayrand, et al., Overview of human papillomavirus-based and other novel options for cervical cancer screening in developed and developing countries, Vaccine 26 (Suppl 10) (2008) K29–K41.
[11] T. Ngoma, R. Muwonge, J. Mwaiselage, J. Kawegere, P. Bukori, R. Sankaranarayanan, Evaluation of cervical visual inspection screening in Dar es Salaam, Tanzania, Int. J. Gynaecol. Obstet. 109 (2) (2010) 100–104.
[12] A. Vallely, Sexually transmitted infections in Papua New Guinea: Achievements, challenges and the way forward. Proceedings of the 50th Annual Symposium of the Medical Society of Papua New Guinea September; Goroka, Papua New Guinea, 2014.
[13] Cancer IAfRo, Biological agents. volume 100 B. A review of human carcinogens, IARC Monogr. Eval. Carcinog. Risks Hum. 100 (Pt B) (2012) 1–441.
[14] J. Dillner, M. Rebolj, P. Birembaut, K.U. Petry, A. Szarewski, C. Munk, et al., Long term predictive values of cytology and human papillomavirus testing in cervical cancer screening: joint European cohort study, Br. Med. J. (Clin. Res. Ed.) 337 (2008) a1754.
[15] R. Sankaranarayanan, B.M. Nene, S.S. Shastri, K. Jayant, R. Muwonge, A.M. Budukh, et al., HPV screening for cervical cancer in rural India, N. Engl. J. Med. 360 (14) (2009) 1385–1394.
[16] D.C. Rijkaart, J. Berkhof, L. Rozendaal, F.J. van Kemenade, N.W. Bulkmans, D.A. Heideman, et al., Human papillomavirus testing for the detection of high-grade cervical intraepithelial neoplasia and cancer: final results of the POBASCAM randomised controlled trial, Lancet Oncol. 13 (1) (2012) 78–88.
[17] Australian Government DoH. National Cervical Screening Program. Overview of the Renewal, 2014 [Available from: 〈http://www.cancerscreening.gov.au/internet/screening/publishing.nsf/Content/overview-of-the-renewal〉.
[18] M.H. Einstein, K.M. Smith, T.E. Davis, K.M. Schmeler, D.G. Ferris, A.H. Savage, et al., Clinical evaluation of the cartridge-based GeneXpert human papillomavirus assay in women referred for colposcopy, J. Clin. Microbiol. 52 (6) (2014) 2089–2095.
[19] K. Canfell, Compass - a randomised controlled trial of HPV screening in Australia. Preventing Cervical Cancer: Integrating Screening and Vaccination (PCC2015 Conference); 20-22 February; Melbourne, 2015.
[20] World Health Organization, WHO Guidelines for Screening and Treatment of Precancerous Lesions for Cervical Cancer Prevention, World Health Organization Press, Geneva, 2013.
[21] J. Cuzick, K. Cuschieri, K. Denton, M. Hopkins, M.A. Thorat, C. Wright, et al., Performance of the Xpert HPV assay in women attending for cervical screening, Papillomavirus Res. 1 (2015) 32–37.
[22] P.E. Castle, B. Eaton, J. Reid, D. Getman, J. Dockter, Comparison of human papillomavirus detection by Aptima HPV and cobas HPV tests in a population of women referred for colposcopy following detection of atypical squamous cells of undetermined significance by Pap cytology, J. Clin. Microbiol. 53 (4) (2015) 1277–1281.
[23] P. Toliman, S.G. Badman, J. Gabuzzi, S. Silim, L. Forereme, A. Kumbia, et al., Field evaluation of xpert HPV point-of-care test for detection of human papillomavirus infection by use of self-collected vaginal and clinician-collected cervical specimens, J. Clin. Microbiol. 54 (7) (2016) 1734–1737.
[24] M. Kunckler, F. Schumacher, B. Kenfack, R. Catarino, M. Viviano, E. Tincho, et al., Cervical cancer screening in a low-resource setting: a pilot study on an HPV-based screen-and-treat approach, Cancer Med. (2017).
[25] P. Toliman, S. Phillips, S.G. Badman, J.M. Kaldor, A. Vallely, S.N. Tabrizi Evaluation of self-collected vaginal specimens fo rthe detection of high-risk HPV infection in a low resource setting. Proceedings of the 31st International Papillomavirus Conference February 28-March 4, 2017 Cape Town, South Africa: International Papillomavirus Conference, 2017.
[26] M.A. Dartell, V. Rasch, T. Iftner, C. Kahesa, J.D. Mwaiselage, J. Junge, et al., Performance of visual inspection with acetic acid and human papillomavirus testing for detection of high-grade cervical lesions in HIV positive and HIV negative Tanzanian women, Int. J. Cancer 135 (4) (2014) 896–904.
[27] P.M. Tebeu, J. Fokom-Domgue, V. Crofts, E. Flahaut, R. Catarino, S. Untiet, et al., Effectiveness of a two-stage strategy with HPV testing followed by visual inspection with acetic acid for cervical cancer screening in a low-income setting, Int. J. Cancer 136 (6) (2015) E743–E750.
[28] Office NS. 2011 National Population and Housing Census: Ward Population Profile - Highlands Region. In: Office nS, editor. Port Moresby: Government of Papua New Guinea; 2014. p. 1-58.
[29] R. Nayar, D.C. Wilbur, The Pap test and Bethesda 2014. "The reports of my demise have been greatly exaggerated." (after a quotation from mark Twain), Acta Cytol. 59 (2) (2015) 121–132.
[30] P. Basu, D. Banerjee, S. Mittal, R. Mandal, I. Ghosh, P. Das, et al., Evaluation of a compact, rechargeable, magnifying device to triage VIA and HPV positive women in a cervical cancer screening program in rural India, Cancer Causes Control 27 (10) (2016) 1253–1259.
[31] C.O. Chigbu, A.K. Onyebuchi, See-and-treat management of high-grade squamous intraepithelial lesions in a resource-constrained African setting, Int. J. Gynaecol. Obstet. 124 (3) (2014) 204–206.
[32] VCS, Victorian Cervical Cytology Registry Statistical Report 2014, Victorian Cytology Service, Melbourne, Australia, 2014.
[33] C. Chiappetta, E. Lendaro, J. Cacciotti, R. Zaralli, C. Puggioni, G. Migliore, et al., Primary HPV test screening in cervical cancer: a two-year experience of a single screening center in Latina (Italy), Eur. J. Gynaecol. Oncol. 36 (5) (2015) 569–573.
[34] S.D. Isidean, M.H. Mayrand, A.V. Ramanakumar, I. Rodrigues, A. Ferenczy, S. Ratnam, et al., Comparison of triage strategies for HPV-positive women: Canadian cervical cancer screening trial results, Cancer Epidemiol. Biomarkers Prev. 26 (6) (2017) 923–929.
[35] R. Luttmer, L.M. De Strooper, R.D. Steenbergen, J. Berkhof, P.J. Snijders, D.A. Heideman, et al., Management of high-risk HPV-positive women for detection of cervical (pre)cancer, Expert Rev. Mol. Diagn. 16 (9) (2016) 961–974.
[36] J.E. Tota, J. Bentley, J. Blake, F. Coutlee, M.A. Duggan, A. Ferenczy, et al., Approaches for triaging women who test positive for human papillomavirus in cervical cancer screening, Prev. Med. 98 (2017) 15–20.
[37] O. Veijalainen, S. Kares, P. Kujala, M. Tirkkonen, R. Vuento, I. Kholova, et al., Human papillomavirus test with cytology triage in organized screening for cervical cancer, Acta Obstet. Gynecol. Scand. 95 (11) (2016) 1220–1227.
[38] K.T. Simms, M. Hall, M.A. Smith, J.B. Lew, S. Hughes, S. Yuill, et al., Optimal management strategies for primary HPV testing for cervical screening: cost-effectiveness evaluation for the National Cervical Screening Program in Australia, PLoS One 12 (1) (2017) e0163509.
[39] J.D. Goldhaber-Fiebert, L.E. Denny, M. De Souza, T.C. Wright Jr, L. Kuhn, S.J. Goldie, The costs of reducing loss to follow-up in South African cervical cancer screening, Cost. Eff. Resour. Alloc.: C./E 3 (2005) 11.
[40] D. Maine, S. Hurlburt, D. Greeson, Cervical cancer prevention in the 21st century: cost is not the only issue, Am. J. Public Health 101 (9) (2011) 1549–1555.
[41] C.J. Chibwesha, B. Frett, K. Katundu, A.C. Bateman, A. Shibemba, S. Kapambwe, et al., Clinical performance validation of 4 point-of-care cervical cancer screening tests in HIV-infected women in Zambia, J. Low. Genit. Tract Dis. 20 (3) (2016) 218–223.
[42] F.H. Zhao, J. Jeronimo, Y.L. Qiao, J. Schweizer, W. Chen, M. Valdez, et al., An evaluation of novel, lower-cost molecular screening tests for human papillomavirus in rural China, Cancer Prev. Res. 6 (9) (2013) 938–948.
[43] P.E. Castle, D. Solomon, A. Hildesheim, R. Herrero, M. Concepcion Bratti, M.E. Sherman, et al., Stability of archived liquid-based cervical cytologic specimens, Cancer 99 (2) (2003) 89–96.

Author/s:
Toliman, PJ;Kaldor, JM;Badman, SG;Gabuzzi, J;Silim, S;Kumbia, A;Kombuk, B;Kombati, Z;Munnull, G;Guy, R;Vallely, LM;Kelly-Hanku, A;Wand, H;Ryan, C;Tan, G;Brotherton, J;Saville, M;Mola, GDL;Garland, SM;Tabrizi, SN;Vallely, AJ

Title:
Performance of clinical screening algorithms comprising point-of-care HPV-DNA testing using self-collected vaginal specimens, and visual inspection of the cervix with acetic acid, for the detection of underlying high-grade squamous intraepithelial lesions in Papua New Guinea

Date:
2018-12

Citation:
Toliman, P. J., Kaldor, J. M., Badman, S. G., Gabuzzi, J., Silim, S., Kumbia, A., Kombuk, B., Kombati, Z., Munnull, G., Guy, R., Vallely, L. M., Kelly-Hanku, A., Wand, H., Ryan, C., Tan, G., Brotherton, J., Saville, M., Mola, G. D. L., Garland, S. M. ,... Vallely, A. J. (2018). Performance of clinical screening algorithms comprising point-of-care HPV-DNA testing using self-collected vaginal specimens, and visual inspection of the cervix with acetic acid, for the detection of underlying high-grade squamous intraepithelial lesions in Papua New Guinea. PAPILLOMAVIRUS RESEARCH, 6, pp.70-76. https://doi.org/10.1016/j.pvr.2018.10.009.

Persistent Link:
http://hdl.handle.net/11343/253123