Epidemiological surveillance of human papillomavirus prevalence and type distribution in Papua New Guinea: the selection of an appropriate laboratory tool

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SUMMARY

Cervical cancer is one of the most common cancers among women worldwide and is a leading cause of cancer death in Papua New Guinea (PNG). It is well established that persistent infection with high-risk types of human papillomavirus (HPV) is necessary for the development of cervical cancer. The recent licensing of two vaccines for the prevention of the two most common high-risk HPV types has prompted renewed interest in the prevention of cervical cancer and HPV in PNG. This review aims to assess and compare available technologies suitable for the epidemiological surveillance of HPV in PNG. Data from the surveillance exercise will provide critical information to the National Department of Health to make an informed decision regarding the introduction of a preventive HPV vaccine.

Introduction

Cervical cancer is one of the most common cancers among women worldwide. It is estimated that there are 530,000 new cases of cervical cancer per year, and approximately 275,000 deaths globally (1). Papua New Guinea (PNG) is estimated to have one of the highest rates of cervical cancer in the world, with an estimated 1500 deaths/year, corresponding to an age-standardized incidence of 40/100,000 (2). It is now well established that persistent infection with certain high-risk types of human papillomavirus (HPV) is necessary for the development of cervical intraepithelial neoplasia (CIN) and cancer of the cervix.

There are over 120 types of HPV and of these an estimated 40 infect the anogenital tract (3). These HPV types are classified as either high-risk (HR) or low-risk (LR) types. The most notable HR-HPV types are type -16 and -18 which are present in 70% of invasive cervical cancers (1,4). The LR-HPV types (for cervical cancer) are responsible for genital warts and include HPV-6 and HPV-11 (3).

Two recombinant HPV vaccines were licensed in 2006 – the quadrivalent Gardasil® vaccine (Merck, New Jersey, USA), which is protective against HR-HPV -16 and -18 and LR-HPV -6 and -11; and the bivalent Cervarix® vaccine (GSK, Middlesex, United Kingdom), which includes HR-HPV -16 and -18 only. Gardasil has been available in the Australian public health system since 2006. The vaccine has been highly effective, as demonstrated by the virtual disappearance of genital warts and significantly lower rates of high-grade cervical abnormalities in the vaccinated population compared to non-vaccinated age cohorts (5,6). Also in Oceania, Gardasil was trialed in Fiji after seminal work found that of 296 cervical cancer and precancerous tissue samples, 99% were positive for an HR-HPV type and of these, 77% were positive for HPV-16 or -18 (7). There are no data concerning HPV prevalence or type distribution in the general population of PNG. A 2009 analysis of cervical biopsies (n = 70) from women with cervical cancer in PNG found that 82.8%
were positive for HR-HPV types 16 (57.1%) and 18 (25.7%) (8). As yet, neither HPV vaccine is available in PNG’s public health system. A key factor restricting the decision to introduce the vaccine to PNG is a lack of robust epidemiological data concerning HPV type distribution in the general population.

Laboratory-based surveillance of the circulating HPV types is required to understand HPV epidemiology within PNG. HPV DNA testing can also be used clinically as an indicator of a woman’s risk of developing cervical cancer. There are many commercial HPV testing kits available and all enable some level of HPV detection or typing at a molecular level. HPV is a small DNA virus of approximately 8000 base pairs with six early (E) genes responsible for replication and transcription and two late (L) structural genes. HPV genotyping is typically performed using molecular tools that most commonly compare all or part of the major capsid protein of the virus encoded by the L1 gene but can be done by targeting the non-structural, regulatory proteins, E6/E7, or using technology employing RNA probes.

Some HPV tests are designed to screen for groups of HR-HPV types only, and will not provide information on specific genotypes, while others are capable of specifically typing all mucosal HR and LR HPV types. The most commonly used clinical assays screen for all HR-HPV types but will only differentiate between the vaccine-specific types, HPV-16 and -18. Some assays utilize standard laboratory equipment only and others require dedicated machinery and a highly sophisticated molecular laboratory. There are a number of HPV tests that have been approved for clinical use by the US Food and Drug Administration (FDA) and the European Union Conformité Européenne (CE), whilst others are designated for research purposes only.

As the PNG Institute of Medical Research (PNGIMR) is currently gearing up to lead a comprehensive program of research on cervical cancer and HPV epidemiology, the wide range of HPV test kits available has prompted us to assess which would be most suitable for HPV surveillance in PNG. The choice of test kit can markedly affect the quality of results generated and as the planned work is the most comprehensive investigation into HPV prevalence and type distribution in the country, the need to generate results that can be standardized internationally is paramount. This review therefore focuses on assessing commercially available HPV testing kits, and specifically compares their genotyping ability, specific technology required and previous performance in other published laboratory-based surveillance programs. This will help to determine the most appropriate HPV test for use in PNG.

Methods

A list of currently available commercial HPV test kits was compiled and adapted from those cited in Poljak and Kocjan (2010) and in Tabrizi (2010) (9,10). Test kits for which we could not identify two or more independent peer-reviewed publications or those for which we could not obtain information from the company website or through communication with the company were not included in this list or considered for use.

Test kits were identified as either being HR-HPV-based screening assays, which refers to those that detect groups of HR-HPV, or HPV genotyping assays, those which allow for individual typing of HR and LR HPV types. Tests were further differentiated based on technology. We compiled information about the required dedicated machinery and the HPV types detected from company-provided product inserts, and assessed the test performance from peer-reviewed publications. The number of peer-reviewed publications concerning each test kit was determined from a search using the test kit name as the key search term in PubMed, and restricted to those concerning genital screening only.

Test kits were shortlisted to be considered useful for epidemiological surveillance in PNG if they met the following three criteria: 1. The test kit could specifically genotype a wide range of HR and LR HPV types; 2. The test kit required only standard laboratory equipment, including a standard or real time thermocycler, centrifuges, water baths, incubators, heating blocks, rotators and plate readers; and 3. There were at least two independent peer-reviewed publications describing its use.

Results

We compiled a list of 11 commercial HPV test kits (Table 1), and from this we identified 7 as HR-HPV-based screening assays and 4
### TABLE 1
**Short list of candidate HPV typing and screening assays**

<table>
<thead>
<tr>
<th>Name</th>
<th>Company</th>
<th>HR HPV</th>
<th>LR HPV</th>
<th>Target</th>
<th>Certifications</th>
<th>Non-standard laboratory equipment</th>
<th>Number of original research papers†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HR-HPV screening assays (some with limited genotyping)</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>RNA:DNA hybridization with chemiluminescent detection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hybrid capture II HPV DNA</td>
<td>Qiagen</td>
<td>16,18,31,33,35,39,45,51,52,56,58,59,68</td>
<td>None</td>
<td>FDA</td>
<td>Dedicated machine</td>
<td>Not yet Dedicated machine (low cost)</td>
<td>&gt;50</td>
</tr>
<tr>
<td>careHPV</td>
<td>Qiagen</td>
<td>16,18,31,33,35,39,45,51,52,56,58,59,68</td>
<td>None</td>
<td>Not yet</td>
<td>Dedicated machine</td>
<td>Not yet Dedicated machine (low cost)</td>
<td>3</td>
</tr>
<tr>
<td>Cervista HPV HR Test</td>
<td>Hologic</td>
<td>16,18,31,33,35,39,45,51,52,56,58,59,66,68</td>
<td>None</td>
<td>FDA</td>
<td>No</td>
<td>No</td>
<td>~20</td>
</tr>
<tr>
<td><strong>Transcription-mediated amplification</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aptima HPV</td>
<td>GenProbe</td>
<td>16,18,31,33,35,39,45,51,52,56,58,59,66,68</td>
<td>None</td>
<td>E6/E7</td>
<td>CE-IVD</td>
<td>Dedicated machine</td>
<td>~15</td>
</tr>
<tr>
<td><strong>PCR with nucleic acid hybridization detection system (PCR-EIA)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplicor HPV Test</td>
<td>Roche</td>
<td>16,18,31,33,35,39,45,51,52,56,58,59,68</td>
<td>None</td>
<td>L1</td>
<td>CE-IVD</td>
<td>No</td>
<td>~30</td>
</tr>
<tr>
<td>COBAS 4800 HPV Test</td>
<td>Roche</td>
<td>16*,18*,31,33,35,39,45,51,52,56,58,59,66,68</td>
<td>None</td>
<td>L1</td>
<td>FDA</td>
<td>COBAS machine</td>
<td>~15</td>
</tr>
<tr>
<td>RealTime High Risk HPV Test</td>
<td>Abbott</td>
<td>16*,18*,31,33,35,39,45,51,52,56,58,59,66,68</td>
<td>None</td>
<td>L1</td>
<td>CE-IVD</td>
<td>m2000 real time PCR system</td>
<td>~15</td>
</tr>
</tbody>
</table>
### Specific HPV genotyping assays

#### Reverse hybridization assays using a microarray detection system

<table>
<thead>
<tr>
<th>Test</th>
<th>Manufacturer</th>
<th>Genotypes</th>
<th>CE-IVD</th>
<th>Microarray Reader</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>PapilloCheck HPV Screening Test</td>
<td>Greiner Bio-One</td>
<td>16,18,31,33,35,39,45,51,52,53,56,58,59,66,68,73,82</td>
<td>E1</td>
<td>CE-IVD</td>
<td>~15</td>
</tr>
<tr>
<td>CLART Human Papillomavirus 2</td>
<td>Genomica</td>
<td>16,18,31,33,35,39,45,51,52,53,56,58,59,66,68,73,82</td>
<td>L1</td>
<td>CE-IVD</td>
<td>2</td>
</tr>
</tbody>
</table>

#### Reverse hybridization using a line blot detection system

<table>
<thead>
<tr>
<th>Test</th>
<th>Manufacturer</th>
<th>Genotypes</th>
<th>CE-IVD</th>
<th>Automation</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>INNO-LiPA HPV Genotyping Test</td>
<td>Innogenetics</td>
<td>16,18,31,33,35,39,45,51,52,53,56,58,59,66,68,73,82</td>
<td>L1</td>
<td>No, but can be</td>
<td>~40</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>automated</td>
<td></td>
</tr>
<tr>
<td>Linear Array HPV Genotyping Test</td>
<td>Roche</td>
<td>16,18,31,33,35,39,45,51,52,53,56,58,59,66,68,73,82</td>
<td>L1</td>
<td>No, but can be</td>
<td>&gt;50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>automated</td>
<td></td>
</tr>
</tbody>
</table>

†restricted to papers concerning genital screening only
*specifically genotypes types 16 and 18
HPV = human papillomavirus
HR = high risk
LR = low risk
RNA = ribonucleic acid
DNA = deoxyribonucleic acid
FDA = United States of America Food and Drug Administration
CE-IVD = Conformité Européenne in Vitro Diagnostics
PCR-EIA = polymerase chain reaction – enzyme immunoassay
as being HPV genotyping assays. In the latter group, all four assays detected the same 17 HR-HPV types, but differed in their ability to detect LR-HPV types. All assays capable of detecting LR-HPV types detected types 6 and 11. Based on our criteria to select a test for epidemiological surveillance of HPV in PNG, the following tests were shortlisted as suitable candidates: Linear Array HPV Genotyping Test (Roche Diagnostics, Mannheim, USA), 37 types including 20 LR types; PapilloCheck HPV Screening Test (Greiner Bio-One, Frickenhausen, Germany), 25 types including 8 LR types; INNO-LiPA (Innogenetics, Gent, Belgium), 28 types including 11 LR types; and CLART Human Papillomavirus 2 (Genomica, Coslada, Spain), 33 types including 16 LR types. For the remainder of this article, these tests will be referred to simply as Linear Array, PapilloCheck, INNO-LiPA and CLART HPV, respectively.

The four suitable candidate assays were reverse hybridization assays. The PapilloCheck and CLART HPV assays utilized DNA microarray technology for the result read-out, and the INNO-LiPA and Linear Array assays used a line blot detection system. Microarrays require a specific machine to analyse the results, and these two assays were therefore deemed unsuitable for use in PNG. The line blot detection assays can be operated using standard laboratory equipment and therefore the suitable candidate assays for HPV surveillance in PNG were the INNO-LiPA and Linear Array.

The INNO-LiPA and Linear Array assays have CE approval as in vitro diagnostics and have been widely published in the international literature. Direct comparisons of these two assays indicate comparable performance in many studies. Specifically, a large study using cervical scrapes (n = 573) found that over 80% of all sample results were concordant between the two assays, and a further 11% were compatible (at least one type matching in multiple infections) (11). Sensitivity has been reported to be higher for INNO-LiPA than Linear Array, although one study reporting this was specifically investigating HPV types in archival tissue samples up to 10 years old, and hypothesized that the shorter amplification target of INNO-LiPA serves as an advantage in this situation (12). In contrast, Sabol et al. (2008) reported that Linear Array was more sensitive than INNO-LiPA in detecting less prevalent HPV types, and was more effective in detecting multiple infections from cervical DNA samples (13). The superior ability of Linear Array to detect multiple genotypes was confirmed in a further study comparing the two assays (14).

Discussion

We have compiled a list of available HPV testing kits and assessed them for use in the PNG context based on the number and types of HPV detected, the specific machinery required and their performance in published studies. The use of an appropriate HPV test kit is imperative for data generation on HPV prevalence and genotype distribution in PNG. These data are required to build upon the evidence base that the National Department of Health needs in order to make informed decisions regarding the introduction of the HPV vaccine into the country.

The genotyping tests that best met our selection criteria were Linear Array and INNO-LiPA. These tests have a wide HPV detection range for HR and LR types, require standard molecular biology tools and have been extensively used in previous investigations. The Linear Array assay has been utilized to type HPV for epidemiological studies in many diverse settings, including, but not limited to, Romania (15), Switzerland (16), China (17), Botswana (18) and Honduras (19). Similarly, INNO-LiPA has been used in studies focused on archived specimens in Sri Lanka (20) and Fiji (7) as well as large epidemiological studies in Thailand (21) and France (22) and the first HPV investigation in Sardinia, Italy (23).

Our choice of test was guided by our purpose and the setting in PNG. Firstly, HPV tests in PNG are currently required for epidemiological surveillance rather than for clinical management and therefore the ability of the test to specifically genotype a large number of HPV types is of critical value. Secondly, only tests requiring standard laboratory equipment were considered as maintenance and support of specialized equipment can be very challenging in PNG. Further, conducting the testing in PNG, rather than opting to send the samples internationally, has a number of advantages, including the growth in capacity of local scientists, laboratory technicians and the laboratory facilities themselves. In addition, managing testing and surveillance in-country will build knowledge in this area of medical research, and facilitate PNG ownership and
use of these data to inform policy regarding the introduction of an HPV vaccine and cervical cancer prevention programs.

Efforts to introduce a vaccine must occur alongside the continual improvement of the cervical cancer screening program in PNG. An effective cervical cancer screening program can reduce the cervical cancer mortality by more than 50% in settings in which it is optimally introduced. The two most commonly accepted screening protocols are cytology (Papanicolaou smear or ‘Pap smear’) and visual inspection of the cervix with acetic acid (VIA).

HPV DNA testing offers a major advance in cervical cancer screening programs, especially in resource-limited settings. HPV testing allows the identification of women who are at higher risk of developing cervical cancer who can then be monitored and treated appropriately, eg, by VIA and cryotherapy. Previously limited by high cost and the need for sophisticated molecular laboratories, HPV testing now promises to become accessible for clinical screening in resource-limited settings with the release of newly developed assays that can be undertaken using closed units in field settings. Such assays are in various stages of development and implementation, but could be very useful in the future by increasing access to cervical cancer screening and early treatment programs in PNG.

In conclusion, our assessment of available HPV testing kits has indicated that there are two wide-range HPV genotyping kits (Linear Array and INNO-LiPA) suitable for use within the HPV and cervical cancer research program in PNG. This program will provide the first data on the distribution of HPV types in a geographically diverse sample within PNG. The results from this work will be imperative to inform decisions regarding the future introduction of a preventive HPV vaccine into PNG.

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