Health Policy Advisory Committee on Technology

Technology Brief

HPV OncoTect™ E6, E7 mRNA assay to guide colposcopy referral in cervical cancer screening

February 2014

HealthPACT
emerging health technology
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This brief was prepared by Tom Vreugdenburg from the Australian Safety and Efficacy Register of New Interventions Procedures – Surgical (ASERNIP-S).
**Technology, Company and Licensing**

Register ID  WP174

Technology name  HPV OncoTect™ E6, E7 mRNA assay

Patient indication  Women with abnormal cervical cytology results from routine cervical cancer screening or positive HPV triage results, prior to referral for colposcopy

**Description of the technology**

The Papanicolaou test, or Pap smear test, is currently used as a primary screening tool to detect pre-cancerous changes or abnormalities in cervical cells before progression to cancer occurs.(1) During a Pap smear test, a speculum is used to open the vaginal canal so that cervical cells can be collected with an endocervical brush. The collected cells are then smeared on a slide for examination under a microscope. To improve sample collection, liquid-based cytology (LBC) techniques have been developed as an alternative to conventional smear cytology. With LBC, cells are collected with a traditional endocervical brush, but are rinsed into a vial with a preservation fluid instead of being smeared directly onto a slide.(2)

The Human Papillomavirus (HPV) OncoTect™ E6, E7 mRNA assay is an in vitro diagnostic (IVD) test used to detect pre-cancerous changes in cervical cells collected during routine liquid-based cervical cytology screening. The test provides a quantitative measure of the expression of the E6 and E7 oncogenes, which produce the oncoproteins responsible for the abnormal cellular changes that lead to cancer.(3) Messenger ribonucleic acid (mRNA) transfers genetic information to the cellular sites of protein synthesis within cells which specify protein products of gene expression. The OncoTect™ assay detects the levels of E6 and E7 mRNA in intact cervical cells, which indicate whether or not the HPV virus has begun to trigger cancerous changes. The test then combines two techniques, called in situ hybridisation and flow cytometry. When combined, these techniques measure the percentage of cells expressing E6, E7 mRNA which gives an indication about potential cancerous transformation, as illustrated in Figure 1.
The test is carried out in six separate steps, as represented in Figure 2.(5) In the first step, cervical cytology specimens are collected and stored in conventional liquid-based cytology vials.(6) The cells are then fixed and made permeable in a one-step reaction, and then washed twice. Probe molecules, which have been marked with fluorescent tracers, are then attached (hybridised) to all E6 and E7 mRNA targets inside the intact cells. The cells are then washed again to remove any unbound or mismatched probe molecules. Finally, the hybridised cells are analysed by flow cytometry, whereby a laser detects the fluorescent signal of each cell. This signal is directly proportional to the amount of target E6 and E7 mRNA in each cell.(7)

The clinical threshold for a positive test result with the HPV OncoTect™ E6, E7 mRNA assay is the over-expression of E6 and E7 mRNA in at least two per cent of the total sample of cells. If less than two per cent of the sample is shown to over-express the E6 and E7 oncogenes, then the result is negative.(5) A positive result may indicate a pre-cancerous stage of the disease, which means that the woman has an increased risk of developing cervical cancer in
the future if she is not treated. In this instance, a colposcopy and, potentially, a biopsy are needed to determine the true disease status of the patient and to inform clinical decisions regarding treatment. A negative result may indicate that the woman is at low risk of developing cervical cancer and can continue with biennial screening.(8)

In terms of clinical utility, the differentiating factor between the HPV OncoTect™ E6, E7 mRNA assay and other mRNA-based assays is its ability to keep the cells intact throughout the testing process. By doing so, the test is able to measure both the expression of E6, E7 mRNA in individual cells and the total proportion of cells that are affected. Thus, the HPV OncoTect™ E6, E7 mRNA assay may also be able to detect the presence of disease, rather than simply the presence of HPV infection like other E6, E7 mRNA assays.

Company or developer
The HPV OncoTect™ E6, E7 mRNA assay is manufactured by IncellDx™, Inc. (Menlo Park, CA, United States of America).

Reason for assessment
The HPV OncoTect™ E6, E7 mRNA assay is a new test for the early detection of cervical cancer. The primary aim of the test is to guide the referral of women with an unsatisfactory or abnormal screening cytology result for additional, more invasive, testing (colposcopy or biopsy, or both). If the HPV OncoTect™ E6, E7 mRNA assay has better diagnostic accuracy than current testing methods, it may reduce the number of women who are needlessly referred for invasive testing.

Stage of development in Australia
- Yet to emerge
- Experimental
- Investigational
- Nearly established
- Established
- Established but changed indication or modification of technique
- Should be taken out of use

Licensing, reimbursement and other approval
The HPV OncoTect™ E6, E7 mRNA assay has received the European IVD/CE mark and is available throughout most of the world. In the United States, the assay is labelled as an analyte specific reagent probe, which is a regulatory category for early-stage IVDs that have not yet been examined by the United States Food and Drug Administration (FDA) (personal communication, IncellDx™).

A summary of the market registration status of HPV E6, E7 mRNA tests in the European Union and the United States is provided in Table 1. None of the identified tests are listed on the Medicare Benefits Schedule (MBS) at the time of writing, due to the current regulatory framework for IVDs. Updated regulatory guidelines for IVDs introduced on 1 July 2010 will
come into effect on 1 July 2014, at which time these devices will be required to seek ARTG inclusion to ensure their continued supply in Australia.(9)

Table 1  HPV E6, E7 mRNA manufacturers and test properties (10-12)

<table>
<thead>
<tr>
<th>Device Name</th>
<th>CE mark and start date</th>
<th>FDA clearance and start date</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV OncoTect™ E6, E7 mRNA assay</td>
<td>IVD/CE</td>
<td>None identified</td>
</tr>
<tr>
<td></td>
<td>Start date: January 2006</td>
<td></td>
</tr>
<tr>
<td>PreTect HPV-Proofer</td>
<td>IVD/CE</td>
<td>None identified</td>
</tr>
<tr>
<td></td>
<td>Start date: May 2003</td>
<td></td>
</tr>
<tr>
<td>APTIMA HPV assay</td>
<td>IVD/CE</td>
<td>Premarket approval: P100042</td>
</tr>
<tr>
<td></td>
<td>Start date: May 2008</td>
<td>Start date: 28 October 2011</td>
</tr>
<tr>
<td>NuclISENS EasyQ® HPV</td>
<td>IVD/CE</td>
<td>None identified</td>
</tr>
<tr>
<td></td>
<td>Start date: not identified</td>
<td></td>
</tr>
</tbody>
</table>


Australian Therapeutic Goods Administration approval

☑ Yes          ARTG number(s) N/A
☒ No
☐ Not applicable

Technology type    Diagnostics
Technology use     Diagnostic

Patient Indication and Setting

Disease description and associated mortality and morbidity

Cervical cancer affects the cells of the uterine cervix. It can arise from the squamous cells that cover the outer surface of the cervix (squamous cell carcinoma) or from the glandular cells in the cervical canal (adenocarcinoma). Abnormal changes in the squamous cells or growth in the layers of the cervix are referred to as cervical intra-epithelial neoplasia (CIN). Low-grade changes are referred to as CIN 1, high-grade changes as CIN 2 and CIN 3. CIN 3 is equivalent to in situ carcinoma.

Infection with certain types of HPV is necessary, but not sufficient, to cause cancer of the cervix.(13) Although almost all cases (over 99.7 per cent) of cervical cancer are associated with the presence of HPV DNA, cervical cancer remains a rare outcome of infection with HPV. Low-grade cervical abnormalities may occur after an acute HPV infection, but approximately 90 per cent of these changes regress without any treatment.(14) In contrast, high-grade abnormalities may occur after persistent HPV infection in approximately 10 to 13
per cent of cases. (1, 14) The progression of precancerous cellular changes to invasive cervical cancer typically occurs over a period of 10 to 20 years, however five per cent of CIN 2 and 12 per cent of CIN 3 lesions will progress within two years if left untreated. (15)

There are currently more than 100 known types of HPV, of which approximately 40 are capable of infecting the anogenital area and are sexually transmitted. There are at least 14 high-risk types of HPV that cause the development of tumours, but the majority of cervical cancers are associated with the HPV genotypes 16, 18, 39, 45 and 73. The two most prevalent high-risk genotypes are HPV18 (60%) and HP16 (20%). (16, 17) In Australia in 2009, 65 per cent of cervical cancers were squamous cell carcinomas, 26 per cent were adenocarcinomas, and other cervical cancer types made up the remainder. (1)

In terms of burden of disease, cervical cancer accounted for 4,100 disability-adjusted life years in Australia in 2012, of which 3,400 years were lost due to premature death and 700 were lost due to disability and ill-health. (18) While the early stages of cervical cancer are often asymptomatic, more advanced stages of the disease can cause vaginal bleeding and discharge, pain during sexual intercourse, and pelvic pain. Due to advances in the treatment and early detection of cervical cancer, 87 per cent of women survive cervical cancer in the first year, and 72 per cent survive up to five years following a diagnosis. (19) The age-standardised mortality rate attributable to cervical cancer is therefore relatively low, and has decreased from 4.0 cases per 100,000 in 1991 to 1.9 cases per 100,000 in 2009. In 2010, 152 Australian women died from cervical cancer. (1, 12)

**Number of patients**

Cervical cancer is the 12th most common cancer affecting Australian women, with 631 new cases of cervical cancer diagnosed in 2009. (1, 18) In 1991, Australia introduced the National Cervical Screening Program (NCSP), with the aim to screen all sexually active women aged between 18 and 69 years for pre-cancerous changes in cervical cells. (20) Since the inception of the NCSP, the age-standardised incidence rate of cervical cancer in Australia has decreased significantly from 13.0 cases per 100,000 in 1991 to 6.7 cases per 100,000 in 2009. For every 1,000 women screened through the NCSP in 2011, 8 had a high-grade (CIN 2 or CIN 3) abnormality detected by histology. (1) A similar trend has been observed in New Zealand, with the age-standardised incidence of cervical cancer decreasing from 12 per 100,000 in 1991 to 7 per 100,000 in 2002. (20) Internationally, the age-standardised incidence of cervical cancer is diverse, and is positively correlated with higher HPV prevalence, lower human development index score, and the absence of a structured cervical cancer screening program. (21) Differences in cervical cancer incidence are observed both within as well as between regions. (22) For example, the relatively low age-standardised incidence in Finland (4.5 per 100,000 in 2008) is partially attributable to its national screening program, whereas Lithuania has a comparatively high incidence rate (21.0 per 1000,000 in 2008) and does not have a national screening program. (23)
Over the two-year period from 2010 to 2011, the NCSP achieved a 59 per cent participation rate, screening 3,641,189 Australian women between the ages of 20 and 69 years. This translates to 2,149,798 cervical cytology tests performed in 2011, of which 96 per cent were conducted for women aged between 20 and 69 years. In comparison, New Zealand achieved a higher participation rate of 75 per cent, with 852,524 women aged between 25 and 69 years being screened from July to December 2010. Of the cytology tests conducted in Australia in 2011, 92 per cent returned a negative result for which no follow-up action was taken, and the women resumed biennial screening. Therefore, the HPV OncoTect™ E6, E7 mRNA assay would not be useful in this population.

However, 42,760 cytology tests (2%) conducted in 2011 were unsatisfactory, and a further 115,026 (6%) returned an abnormal result. An unsatisfactory result indicates that the sampled cells could not be used to make a cytological diagnosis, while an abnormal result indicates either low-grade (CIN 1), high-grade (CIN 2, CIN 3), or cancerous changes of the cervical cells. Of the 157,786 women with either an unsatisfactory or an abnormal smear result, 75,589 were referred for cervical histology (biopsy) to confirm the diagnosis (3.7 histology tests for every 100 cytology tests conducted in women aged 20 to 69 years). However, 36,117 of these histology tests (48%) returned a negative result. Given the high rate of negative histology results following referral from conventional smear cytology, the HPV OncoTect™ E6, E7 mRNA assay could reduce the number of referrals for biopsy with histology if it has better diagnostic accuracy than conventional triage methods.

Speciality | Obstetrics and Gynaecology
Technology setting | Specialist Hospital

Impact

Alternative and/or complementary technology

The HPV OncoTect™ E6, E7 mRNA assay is primarily used as a triage test to guide colposcopy or biopsy referral for women with ambiguous or abnormal cytology test results from primary screening. In this regard, the HPV OncoTect™ assay may either be used either in combination with, or as a replacement for, other triage tests. These tests include HPV DNA testing or repeat cytology (LBC or conventional).

Current technology

In Australia, conventional Pap smear cytology is currently used as a primary screening tool to detect cervical cancer. While problematic, it has been the backbone of the most successful cancer reduction program in the Australian health system. The main limitation of the Pap smear test is its sensitivity, which is estimated to range from 58 to 96 per cent based on data provided by the Medical Services Advisory Committee (MSAC). In contrast, the specificity of the Pap smear test is higher, ranging from 96 to 98 per cent.
Variability in the diagnostic accuracy of the Pap smear test can be attributed to poor quality sample collection and preparation, and errors in interpretation.

Australian women who return an equivocal cytology result are invited to have a repeat Pap smear test in 6 to 12 weeks, whereas women with an abnormal result indicating either a CIN 2 or CIN 3 lesion are immediately referred for colposcopy. While there are currently several different types of HPV tests available in Australia that target either HPV DNA or mRNA, they are not publicly funded through Medicare for triage testing in cervical cancer screening. Currently, the only publicly funded use for HPV testing in Australia is as a test-for-cure following the treatment of invasive cervical cancer (MBS item 69418).

Women who would like to take advantage of an HPV triage test to better inform their need for colposcopy or biopsy, or both, have a number of options which can be purchased out-of-pocket. The Hybrid Capture 2 High-Risk HPV DNA Test (Digene Corporation, Gaithersburg, MD, USA) has diffused within Australia to a limited extent for use as an out-of-pocket triage test to inform referral for colposcopy and biopsy after an abnormal conventional or liquid-based cytology test result. Other IVDs that test for HPV E6, E7 mRNA expression are outlined in Table 2. The Aptima assay, the NucliSens EasyQ® HPV assay and the PreTect HPV-Proofer utilize E6, E7 gene targets that may or may not be different targets than the HPV OncoTect™ E6, E7 mRNA assay. The APTIMA HPV assay is currently used in two laboratory clinics in Australia as a triage test to inform referrals for colposcopy and is paid for out-of-pocket (personal communication, Hologic GenProbe).

Table 2  HPV E6, E7 mRNA assay manufacturers and test properties (5, 10, 11, 27)

<table>
<thead>
<tr>
<th>Device Name</th>
<th>Manufacturer</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV OncoTect™ E6, E7 mRNA assay</td>
<td>IncellDx™, Inc. Mentlo Park, CA, USA</td>
<td>Quantitative mRNA expression: E6, E7 HPV types: All Method: In situ hybridisation and flow cytometry</td>
</tr>
<tr>
<td>APTIMA HPV assay</td>
<td>Hologic Gen-Probe, Inc. San Diego, CA, USA</td>
<td>Qualitative mRNA expression: E6, E7 HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 Method: In vitro nucleic acid amplification</td>
</tr>
<tr>
<td>NucliSens EasyQ® HPV assay</td>
<td>bioMérieux, Inc. North Carolina, USA</td>
<td>Qualitative mRNA expression: E6, E7 HPV types: 16, 18, 31, 33, 45 Method: Real-time NASBA</td>
</tr>
<tr>
<td>PreTect HPV-Proofer</td>
<td>NorChip AS Kokkarstua, Hurum, Norway</td>
<td>Qualitative mRNA expression: E6, E7 HPV types: 16, 18, 31, 33, 45 Method: Real-time NASBA</td>
</tr>
</tbody>
</table>

HPV: human papillomavirus. NASBA: nucleic acid sequence based amplification.

Diffusion of technology in Australia

The HPV OncoTect™ E6, E7 mRNA assay is not currently included on the ARTG, making it difficult to determine its availability in Australia. Internet searches and personal
communication with the manufacturer were unable to identify any locations in Australia or New Zealand where the test is used in clinical practice.

**International utilisation**

Clinical trials of the HPV OncoTect™ E6, E7 mRNA assay have been completed in several countries (Table 3). The device is currently IVD/CE marked and available for use in the United Kingdom and the European Union.

<table>
<thead>
<tr>
<th>Country</th>
<th>Trials underway or completed</th>
<th>Limited use</th>
<th>Widely diffused</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canada</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Europe</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>South Africa</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>USA</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Zealand</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Cost infrastructure and economic consequences**

Medicare-funded HPV DNA testing in Australia (MBS item number 69418) is currently limited to patients who have received treatment for high-grade squamous intra-epithelial lesions (HSIL) of the cervix within the previous two years, have had a positive HPV test after treatment for HSIL within the previous two years, or are undergoing annual cytological review following treatment for HSIL. Between July 2012 and 2013, high-risk HPV DNA testing for these uses (MBS item 69418) was carried out 34,714 times with a fee of $63.55 per test.

Between July 2012 and June 2013, there were 8,218 colposcopy procedures conducted in private hospitals in Australia (MBS items 35644, 35645, 35646, 35647, 35648), with the majority of claims (6,643 colposcopies) being made on item 35647 ($203.65 per claim).

As the HPV OncoTect™ E6, E7 mRNA assay is not currently funded by Medicare, patients are required to pay for the test at a cost that is approximately equivalent to HPV DNA testing. However, the exact cost to Australian consumers was not available. Currently available HPV DNA and HPV E6, E7 mRNA tests carry co-payment between $20 and $25, which is a good indication of the likely cost of the OncoTect™ assay to consumers (personal communication, Hologic Gen-probe Australia). In comparison, a conventional Pap smear test is publicly funded for a fee of $19.60 (MBS items 73053, 73055, 75057). In the United States, the total cost of the test is US$90.72 plus a consultation fee of US$23.45 (personal communication, IncellDX™ Inc).
Ethical, cultural or religious considerations

The psychosocial stress caused by the confirmed diagnosis of a sexually transmitted infection should be carefully considered when administering a triage test as part of a broader screening program. This may be caused by difficulties in disclosing the presence of a sexually transmitted infection to current or future sexual partners, or a lack of understanding about the implications of the test results. (28, 29) Recent research suggests that young women, in particular, may be likely to interpret a positive HPV test result as a presentiment of cancer and death in the absence of guidance on how to interpret the test results. (30) These issues highlight the importance of providing patients with diagnostic technologies within established systems that provide adequate follow up and standardised patient education.

Evidence and Policy

Safety and effectiveness

The literature search identified five studies that investigated the use of the HPV OncoTect™ E6, E7 mRNA assay for the early detection of cervical cancer. (3, 7, 31-34) Two of these studies, Kottaridi et al. 2011 and Spathis et al. 2013, reported results from the same population, in which case only the most recent was considered. (33, 34) The three studies with the highest level of evidence were included in this Technology Brief (Table 4). Two of these studies enrolled patients who had already received a referral for colposcopy, while the third study by Coquillard et al. 2011 enrolled patients with abnormal cytology results. (31) Non-English language studies were excluded from the literature search.

Safety

Any diagnostic procedure carries a number of safety consequences beyond the risk of physical harm caused by the test being conducted. Specifically, the implications of the test results can be significant and, therefore, need to be carefully considered before the test is implemented in clinical practice. These potential concerns can include the risk of overdiagnosis and treatment of clinically irrelevant disease, the psychosocial effects of positive results and the clinical consequences of false negative test results. There is also the consequence of having a true HPV-positive result, which may cause anxiety and distress even though the infection frequently clears up without progressing to more serious lesions. None of the identified studies reported adverse events relating to these criteria. However, the potential for harm to arise through these routes should not be disregarded. No adverse events were reported during the collection of cervical cell samples in any of the studies.
Table 4  Profile of included diagnostic accuracy studies

<table>
<thead>
<tr>
<th>Study Details</th>
<th>Pierry et al. 2012(3)</th>
<th>Spathis et al. 2012(34)</th>
<th>Coquillard et al. 2011(31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level of evidence</td>
<td>III-2 (diagnostic)*</td>
<td>III-2 (diagnostic)*</td>
<td>III-2 (diagnostic)*</td>
</tr>
<tr>
<td>Study location</td>
<td>USA</td>
<td>Greece</td>
<td>Spain and USA</td>
</tr>
<tr>
<td>Number of patients</td>
<td>n=246</td>
<td>n=1,173</td>
<td>n=260</td>
</tr>
<tr>
<td>Study population</td>
<td>Women aged 19–75 years referred for colposcopy following either cervical cytology and/or HPV DNA testing</td>
<td>Women aged 19–81 years referred for colposcopy based on abnormal cervical cytology</td>
<td>Women of unknown age from multiple recruitment sites, with abnormal cervical cytology results</td>
</tr>
<tr>
<td>Index test(s)</td>
<td>HPV OncoTect™ E6, E7 mRNA assay</td>
<td>HPV OncoTect™ E6, E7 mRNA assay</td>
<td>HPV OncoTect™ E6, E7 mRNA assay</td>
</tr>
<tr>
<td>Comparator(s)</td>
<td>Repeat liquid-based cytology</td>
<td>CLART® HPV2 DNA kit</td>
<td>Hybrid Capture 2 HPV DNA test</td>
</tr>
<tr>
<td>Reference test</td>
<td>Biopsy with histology</td>
<td>Biopsy with histology</td>
<td>Biopsy with histology</td>
</tr>
<tr>
<td>Blinding of index test to reference test results</td>
<td>Unclear</td>
<td>Unclear†</td>
<td>Unclear</td>
</tr>
<tr>
<td>Losses to follow-up</td>
<td>Not reported</td>
<td>Not reported</td>
<td>n=45</td>
</tr>
<tr>
<td>Conflict of interest</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
</tbody>
</table>

*Level III-2 diagnostic evidence denotes a study in which it was not possible to determine whether patient enrolment was consecutive, or if the comparison between the index test and reference standard was blinded.
†Interpretation of the index test was made without reference (blinded) to the results of the comparator tests, and vice versa.

Diagnostic Accuracy

Spathis et al. 2012 (34)

This multi-centre, level III-2 diagnostic accuracy study compared the clinical performance of the HPV OncoTect™ E6, E7 mRNA assay with the CLART® HPV2 DNA assay and liquid-based cytology. Study participants (n=1,173) were aged between 19 and 81 years and had been referred to one of two colposcopy clinics based on a prior abnormal cytology result. No other inclusion criteria or demographic data were reported.

The primary outcomes were sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), stratified by histological diagnosis. Diagnostic accuracy data were calculated by comparing the results of the index and the comparator tests against the reference standard – biopsy with histological confirmation. While the index and comparator tests were interpreted independently of each other, it was not clear whether the investigators who carried out the index test were blinded to the results of the reference test, and vice versa, which potentially introduces test review bias into the study results.(35)
**Efficacy**

In total, punch biopsy identified 597 positive samples; the remaining 576 samples returned both negative repeat cytology and colposcopy results. The performance of the HPV OncoTect™ E6, E7 mRNA assay and the CLART® HPV2 DNA assay, stratified by biopsy stage, are summarised in Table 5. Fourteen HPV DNA test results and 36 OncoTect test results were inadequate, and are not included in Table 5. Combined repeat cytology and colposcopy were used as comparators, but the authors did not verify disease status for the negative results, and therefore the diagnostic accuracy of these tests may be inflated by verification bias. As a result, when lesions equal to or higher grade than atypical squamous cells of unknown significance (ASC-US) were used as a cut-off, cytology reported the highest sensitivity (97%) and negative predictive value (99%) of all the tests being examined.

Using CIN 2 grade lesions or higher as a clinical cut-off, the HPV OncoTect™ E6, E7 mRNA assay demonstrated improved sensitivity (86% vs. 56%) compared with the CLART® HPV2 assay for HPV types 16 and 18, and improved specificity (82% vs. 52%) and PPV (49% vs. 28%) over the CLART® HPV2 DNA assay for all included HPV types. The NPV of all three tests was comparable (Table 6). The positive likelihood ratios for the OncoTect™ assay and CLART® HPV2 assay were 4.7 and 1.9, respectively, indicating that the OncoTect™ assay may be better at detecting high grade dysplasia.

**Table 5**

<table>
<thead>
<tr>
<th>Study</th>
<th>Test Result</th>
<th>CIN 1</th>
<th>CIN 2</th>
<th>CIN 3</th>
<th>SCC</th>
<th>AIS</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spathis et al. 2012 (34)*</td>
<td>OncoTect +ve</td>
<td>82</td>
<td>17</td>
<td>80</td>
<td>76</td>
<td>68</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>OncoTect -ve</td>
<td>489</td>
<td>118</td>
<td>167</td>
<td>7</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>HPV DNA +ve</td>
<td>233</td>
<td>62</td>
<td>174</td>
<td>81</td>
<td>75</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>HPV DNA -ve</td>
<td>336</td>
<td>76</td>
<td>81</td>
<td>9</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Coquillard et al. 2011 (31)</td>
<td>OncoTect +ve</td>
<td>N/A</td>
<td>12</td>
<td>21</td>
<td>36</td>
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<td>8</td>
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<tr>
<td></td>
<td>OncoTect -ve</td>
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<td>97</td>
<td>57</td>
<td>7</td>
<td>2</td>
<td>0</td>
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<tr>
<td></td>
<td>HPV DNA +ve</td>
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<td>57</td>
<td>64</td>
<td>38</td>
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<td>7</td>
</tr>
<tr>
<td></td>
<td>HPV DNA -ve</td>
<td>N/A</td>
<td>52</td>
<td>14</td>
<td>5</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Pierre et al. 2012 (3)†</td>
<td>OncoTect +ve</td>
<td>N/A</td>
<td>7</td>
<td>14</td>
<td>21</td>
<td>19</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>OncoTect -ve</td>
<td>N/A</td>
<td>130</td>
<td>50</td>
<td>4</td>
<td>1</td>
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</tr>
<tr>
<td></td>
<td>Cytology +ve</td>
<td>N/A</td>
<td>129</td>
<td>62</td>
<td>23</td>
<td>19</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Cytology -ve</td>
<td>N/A</td>
<td>8</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>N/A</td>
</tr>
</tbody>
</table>

*Biospy was not conducted on 576 samples which were deemed to be clinically negative, following both a negative cytology and colposcopy result. Fourteen HPV DNA test results and 36 OncoTect test results were inadequate, and are not included in the table.

†Cut-off for positive cytology result included atypical squamous cells of undetermined significance, low-grade squamous intraepithelial lesions, and high-grade squamous intraepithelial lesions.

Table 6  Diagnostic accuracy of HPV OncoTect™ E6, E7 mRNA assay compared with HPV DNA testing or cytology, or both *

<table>
<thead>
<tr>
<th>Study</th>
<th>Test</th>
<th>Sensitivity (n)</th>
<th>Specificity (n)</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spethis et al. 2012 (34)</td>
<td>OncoTect</td>
<td>85.8 (169/197)</td>
<td>81.6 (797/976)</td>
<td>48.6</td>
<td>96.6</td>
</tr>
<tr>
<td></td>
<td>CLART HPV2</td>
<td>92.4 (182/197)</td>
<td>51.9 (507/976)</td>
<td>27.9</td>
<td>97.1</td>
</tr>
<tr>
<td></td>
<td>CLART HPV2 16 or 18</td>
<td>55.8 (110/197)</td>
<td>87.1 (850/976)</td>
<td>46.6</td>
<td>90.7</td>
</tr>
<tr>
<td></td>
<td>LBC for ASCUS+</td>
<td>97.0 (191/197)</td>
<td>67.4 (668/976)</td>
<td>99.1</td>
<td>99.1</td>
</tr>
<tr>
<td></td>
<td>Colposcopy for HSIL+</td>
<td>71.6 (141/197)</td>
<td>96.1 (939/976)</td>
<td>78.8</td>
<td>94.4</td>
</tr>
<tr>
<td>Coquillard et al. 2011 (31)</td>
<td>OncoTect</td>
<td>87.4 (64/73)</td>
<td>82.4 (154/187)</td>
<td>65.9</td>
<td>94.4</td>
</tr>
<tr>
<td></td>
<td>HPV DNA</td>
<td>89.0 (65/73)</td>
<td>35.3 (66/187)</td>
<td>34.9</td>
<td>89.0</td>
</tr>
<tr>
<td>Pierry et al. 2012 (3)†</td>
<td>OncoTect</td>
<td>88.9 (40/45)</td>
<td>88.5 (180/201)</td>
<td>65.6</td>
<td>97.2</td>
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<tr>
<td></td>
<td>LBC for ASCUS+</td>
<td>93.3 (42/45)</td>
<td>5.0 (10/201)</td>
<td>18.0</td>
<td>76.9</td>
</tr>
</tbody>
</table>

*A histology endpoint of CIN2+ was used as a threshold for a positive test in all data reported in this table.
†High-grade squamous cell intraepithelial lesions were used as the cut-off for a positive cytology result.

Coquillard et al. 2011 (31)

Coquillard and colleagues conducted a retrospective, level III-2 diagnostic accuracy study which investigated the relative performance of the HPV OncoTect™ E6, E7 mRNA assay and the Hybrid Capture 2 HPV DNA assay in detecting early-stage cervical cancer. It was stated that pre-existing cervical samples were collected from multiple sites that included both low- and high-risk populations; however, information about the sample population demographics, collection sites, and inclusion criteria were not reported. In total, 2,094 cervical samples were collected, of which 2,049 were deemed suitable. Testing the suitability of samples was conducted prior to investigation with the index tests and does not reflect upon the technical limitations of the test, but rather the method used to collect the cervical samples. A total of 125 cytology results were unaccounted for in the analysis of this study, and may represent technical failures or ambiguous test results.

While the primary sample was not representative of a subgroup of women with abnormal cervical cytology results received both the Hybrid Capture 2 HPV DNA assay as well as the HPV OncoTect™ E6, E7 mRNA assay. This subgroup represents the target population for the OncoTect™ assay as a triage test, and is the focus for this brief. The primary outcome measures reported in the study were sensitivity, specificity, PPV and NPV. Diagnostic accuracy data were collected by comparing the results of the HPV OncoTect™ E6, E7 mRNA assay and the Hybrid Capture 2 DNA assay with histological confirmation. However, it was unclear whether the interpretation of the index test was conducted by assessors who were blinded to the results of the reference test.
**Efficacy**

Measures of diagnostic accuracy were calculated based on a sample of 260 abnormal cytology samples, which were histologically-confirmed with biopsy. In this population, the sensitivity of the HPV OncoTect™ E6, E7 mRNA assay in CIN 2 grade lesions and higher was comparable to the Hybrid Capture 2 HPV DNA assay, as shown in Table 6. However, the OncoTect™ assay demonstrated significantly improved specificity, PPV, and NPV compared with the DNA assay. In the sample that tested negative on cytology (n=1789), the specificity of the OncoTect™ assay was 96 per cent, compared with 78 per cent for the Hybrid Capture 2 HPV DNA assay. However, in practice negative cytology samples would not be tested with the OncoTect™ assay, as there is no need to refer them for colposcopy.

Pierry et al. 2012 (3)

This prospective level III-2 diagnostic accuracy study compared the ability of the HPV OncoTect™ E6, E7 mRNA assay to detect CIN 2+ lesions (that is, grade CIN 2 or higher) to that of repeat cervical cytology. An initial sample of 3,133 women aged between 19 and 75 years was recruited from a high-risk urban screening population, of which 1,088 were at least 30 years of age. Further details about the study inclusion criteria, setting and sample demographics were not reported. All participants received routine cervical cytology either alone or in combination with HPV DNA testing, and were then referred for colposcopy and biopsy where indicated. Of the initial sample, a sub-group of 246 women who were referred for colposcopy and biopsy were included in the final data analysis. As with the previous two studies, the primary outcome measures were sensitivity, specificity, PPV and NPV. It was unclear whether the assessors interpreting the results of the index test were blinded to the reference test results.

**Efficacy**

When CIN 2+ was considered as a clinical cut-off for a positive test result, the HPV OncoTect™ E6, E7 mRNA assay demonstrated lower clinical sensitivity (89% vs. 93%), but higher specificity (90% vs. 5%) and NPV (97% vs. 77%) than repeat liquid-based cytology (Table 6). In women 30 years of age or older, the OncoTect™ assay had identical sensitivity (89%), but much higher specificity (92% vs. 8%) and PPV (71% vs. 16%) compared to repeat cytology in CIN 2+ lesions. In women younger than 30 years, the OncoTect™ assay had lower sensitivity than cytology (88% vs. 96%), but significantly higher specificity (88% vs. 2%) and PPV (64% vs. 19%). The results of this study indicated that the HPV OncoTect™ E6, E7 mRNA assay may be of significant clinical utility in detecting high-grade lesions, owing to its high specificity compared with cytology.

**Economic evaluation**

There were no economic evaluations identified on the use of HPV OncoTect™ E6, E7 mRNA assay for the early detection of cervical cancer.
Ongoing research

An internet-based search of ClinicalTrials.gov and the Australian and New Zealand Clinical Trials Register did not identify any ongoing trials involving the HPV OncoTect™ E6, E7 mRNA assay.

Other issues

In 2005, the National Health and Medical Research Council released updated guidelines for the management of women with irregular screening results through the NCSP. These guidelines recommend repeat Pap testing at 12 months following the detection of a low-grade squamous abnormality, instead of an immediate referral for colposcopy and treatment. The updated guidelines do not recommend the use of HPV triage testing of low-grade squamous intraepithelial lesions, thereby limiting the potential use of a comparative triage test such as the HPV OncoTect™ E6, E7 mRNA assay. Instead, the guidelines recommend less intervention for women with low-grade squamous intraepithelial lesions, giving the HPV infection an opportunity to resolve without treatment.

In addition, a vaccine against HPV types 6, 11, 16 and 18 was introduced through the National Immunisation Program for girls aged 12 to 13 years in 2007. The vaccine is expected to reduce the incidence and mortality rates attributable to cervical cancer, with preliminary evidence demonstrating a decline in both genital warts and cervical abnormalities caused by HPV infection among young Australian women. As a result, the additional utility of HPV triage testing in women who are vaccinated against HPV types 6, 11, 16 and 18 is questionable. However, not all cases of cervical cancer are caused by these subtypes, the OncoTect™ assay is able to test for all types of HPV, and there remain a large proportion of the population that are not vaccinated.

Finally, LBC is currently being considered for MBS listing in Australia by the MSAC. As the HPV OncoTect™ E6, E7 mRNA assay uses cells sampled with LBC and not Pap smear testing, the switch from a primary screen with Pap smear to LBC could negate the need for a duplicate sample, and could therefore reduce the overall cost of implementing the test as part of the NCSP.

Summary of findings

There is mounting evidence that women presenting with an abnormal Pap smear test result may benefit from an additional HPV triage test. Current clinical practice for HPV testing is limited to DNA testing, which measures the presence or absence of HPV infection. In contrast, the HPV OncoTect™ E6, E7 mRNA assay can quantify both the level of expression of E6, E7 oncogenes within individual cells, and the total proportion of cells exhibiting pre-neoplastic change. Therefore, the test is able to identify the presence of cervical dysplasia, instead of merely the presence or absence of HPV infection. This may provide helpful
information for determining which women with abnormal or equivocal Pap smear results require further invasive testing and treatment.

Three low-quality diagnostic accuracy studies investigating the use of the HPV OncoTect™ E6, E7 mRNA assay for detecting cervical cancer showed promising results. In these preliminary studies, the OncoTect™ assay demonstrated reasonable sensitivity and specificity in diagnosing women with cervical abnormalities, either meeting or exceeding that of current HPV DNA-based triage testing and repeat liquid-based cytology. Unlike DNA-based tests, the OncoTect™ assay is able to detect infections caused by all subtypes of HPV, which allows it to better classify women with cervical abnormalities that are not related to high-risk HPV subtypes 16 and 18. This aspect will also become more important in the future, as the proportion of cervical dysplasia caused by other high risk HPV virus subtypes will increase due to the prophylactic vaccination against HPV 16 and 18. Studies with correct populations of patients with abnormal smear test results that look at the effects of the test on clinical decisions regarding treatment and further intervention are needed to truly determine the value of this test for triage.

HealthPACT assessment

Based on the current level of evidence on its diagnostic accuracy, the HPV OncoTect™ E6, E7 mRNA assay may prove to have better clinical utility for the triage of abnormal cytology results for colposcopy than HPV DNA testing and repeat LBC.

The HPV OncoTect™ E6, E7 mRNA assay is one specific test in a range of tests which may be considered for inclusion in Australia’s cervical cancer screening program. This report can contribute to the ongoing debate around HPV triage testing in Australia, but the decision to include an additional test in the NCSP is beyond the responsibility of HealthPACT. It is recommended that this brief be sent to the NCSP and MSAC for consideration, and that further assessment of the technology by HealthPACT is no longer warranted.

Number of studies included

All evidence included for assessment in this Technology Brief has been assessed according to the revised NHMRC levels of evidence. A document summarising these levels may be accessed via the HealthPACT web site.

Total number of studies: 3

Total number of Level III-2 (diagnostic) studies: 3

Search criteria to be used (MeSH terms)

Text search: (HPV AND E6 AND E7 AND mRNA AND sensitivity AND specificity) OR OncoTect OR PreTect OR NucliSens OR EasyQ OR Aptima.

References


20. NHMRC (2005). *Screening to prevent cervical cancer: guidelines for the management of asymptomatic women with screen-detected abnormalities*, National Health and Medical Research Council, Canberra, Australia


26. Medical Services Advisory Committee (2012). *Cell Enrichment Liquid-Based Cytology*, MSAC, Department of Health, Canberra


