HPV OncoTect® E6 E7 mRNA Assay to guide colposcopy referral in cervical cancer screening

HPV OncoTect® E6 E7 mRNA Assay is a laboratory test (based on in situ hybridisation and flow cytometry) that may improve the accuracy with which women who are at increased risk of developing cervical cancer are identified following routine screening. The test detects how much of a marker for early pre-cancerous changes, called HPV E6 E7 mRNA, is present in whole cells collected from the woman’s cervix during screening. If the test is more accurate than current methods at identifying which women need further investigation, it could reduce the number of women who need to undergo invasive procedures such as colposcopy and biopsy.

Background

Cervical cancer is the twelfth most common cancer in women in the UK. In 2008, there were around 8.7 new cases of cervical cancer diagnosed per 100,000 of population, which equates to 2,938 new cases in the UK. A GP will see approximately one new patient every five years. In 2004-06 more than 80% of patients survived beyond one year, and 64% survived beyond five years in England. In 2008, 827 women died from the disease in England and Wales.

Human papilloma virus (HPV) is the major cause of cervical cancer. In most women, HPV infection clears up on its own and causes no harm. However, in a minority of women who have particular high risk types of the HPV virus, changes in the cervical cells are triggered which can develop into cervical cancer if they are not treated.

Current Practice

The NHS Cervical Screening Programme invites women between the ages of 25 and 64 for regular cervical screening every three to five years (depending on their age). This is intended to pick up any abnormal changes in the cervical cells (known as dyskaryosis) that could develop into cancer if left untreated. The cervical screening ‘smear’ test involves a nurse or doctor taking a sample of cells from the surface of the cervix (neck of the womb) with a small brush and placing them in a liquid. The sample is then sent to the laboratory and processed there with the cells being examined under a microscope. This is called cervical cytology testing. In 2010-11, around 3.4 million women were tested (some more than once) and around 3.7 million cervical cytology tests were performed.

Borderline changes or mild dyskaryosis in the cervical cells are found in about 1 in 20 women (5.4% in England, 2010-11), and these women may be then referred for a colposcopy examination. In some parts of the country, a second test is done on the cervical screening sample to see if high risk HPV is present before a referral for colposcopy is made. This second test, called HPV triage, is intended to help filter out
In 2010-11, 68,918 women were referred for colposcopy with screening results of borderline changes or mild dyskaryosis in England. Only 15-20% of these women were found to have an abnormality significant enough to need treatment, the other 80-85% (approximately 55,100-58,600) were probably false alarms, but may need more frequent screening check-ups for a while.

**New Technology**

**IncellDx Inc’s** HPV OncoTect® E6 E7 mRNA Assay combines two techniques called *in situ* hybridisation and flow cytometry. It detects the levels of a molecular marker called HPV E6 E7 mRNA in whole cervical cells in routine liquid-based screening samples, which indicate whether or not the virus has become active in triggering the disease process. In technical terms, the test works by hybridising fluorescently-bound molecular probes to all mRNA targets inside intact cervical cells. A flow cytometer then reads the fluorescent signal of each cell using a laser, which corresponds directly to the amount of target E6 E7 mRNA present in the cell. A positive result may indicate that a pre-cancerous stage of the disease has started, which would put the women at increased risk of developing cervical cancer in the future if she is not treated, and means that a colposcopy (and maybe treatment) is needed. A negative result may indicate that the woman is at low risk and may only need more frequent screening check-ups for a while.

Currently used HPV triage tests detect viral DNA to tell us whether a high risk infection is present or not. There are also several commercially available tests that instead of detecting viral DNA, detect E6 E7 mRNA as a marker of high risk HPV infection, to find out whether the infection may be dormant (harmless) or active (potentially harmful). Examples of such HPV mRNA tests include the PreTect Proofer (from NorChip AS), the NucliSENS EasyQ HPV (from bioMérieux) and the APTIMA HPV assay (from Gen-Probe). Recent systematic reviews of the evidence suggest that mRNA-based tests may help reduce the number of women who need to undergo colposcopy, although further research is needed to confirm this.

What appears to set the HPV OncoTect® E6 E7 mRNA Assay apart from other mRNA-based tests is that it keeps the cells intact, it can measure the quantity of the marker in each cell and also the proportion of cells affected (i.e. how active the virus is in stimulating the disease process). According to the company, with the other DNA and mRNA tests the cervical cells get broken up during processing and they suggest that this may limit them to the detection of high risk infection instead of measuring disease. The company claim that the HPV OncoTect® E6 E7 mRNA Assay may provide a much more accurate way of working out who needs to undergo colposcopy and who does not, because it can detect the presence of disease rather than simply the presence of infection.

The company claim that for women of all ages, the HPV OncoTect® E6 E7 mRNA Assay offers the high specificity (the percentage of correct negative results) needed for it to be useful in confirming either abnormal cervical cytology results or positive HPV triage results, to make referral to colposcopy more selective. Furthermore, the company claim that their test also has high sensitivity (the percentage of correct positive results) that is comparable to currently available HPV triage tests, and could replace high risk HPV DNA testing entirely. For women who do need a colposcopy, the HPV OncoTect® E6 E7 mRNA Assay results may also be useful in guiding the doctor to precisely where in the cervix the biopsy sample should be taken from, based on distinguishing different cells types from the inner and outer surfaces of the cervix.

Looking forwards, the company have recently developed a ‘3Dx’ version of HPV OncoTect® E6 E7 mRNA Assay which can produce automated 3D digital images of intact cells. The E6 E7 mRNA and nuclear staining will be quantified digitally for all analysed cells, and a molecular snapshot of each cell will be
Several observational clinical studies have been published that estimate the accuracy of the HPV OncoTect® E6 E7 mRNA Assay by measuring how well its results agree with cervical abnormality findings confirmed at biopsy. Some of the studies also compare the HPV OncoTect® E6 E7 mRNA Assay results with those from high risk HPV testing.

In a published American and Spanish study, 260 biopsies were performed following the screening of 2,049 routine cervical samples (the women's ages were not stated). The biopsy results showed that of the 260 women, 109 had no cervical abnormality, 78 had mild changes to the cells within the cervix known as CIN1 (CIN stands for cervical intraepithelial neoplasia) and therefore were not at increased risk of developing cervical cancer, 43 had moderate changes known as CIN2, and the remaining 30 had more severe changes known as CIN3, or worse. The accuracy of the HPV OncoTect® E6 E7 mRNA Assay was compared with standard high risk HPV testing using the Hybrid Capture 2 test (from Qiagen). It was found that although the two tests had similar accuracy in correctly identifying women who were at increased risk of developing cervical cancer, the HPV OncoTect® E6 E7 mRNA Assay was more accurate in identifying those women who were not at increased risk, giving the correct ‘all clear’ result (as confirmed by biopsy) in 85% of cases as compared to 35% of cases using standard testing.

In another American study (published as a conference abstract), 200 biopsies were performed following screening of 3,133 cervical samples (from women aged 19-75). It was found that the HPV OncoTect® E6 E7 mRNA Assay correctly identified 69% of women whose biopsy results confirmed that they had CIN2 and 89% of women who had CIN3, and it also correctly identified 94.5% of women who did not have either (i.e. had CIN1 or were normal). In women aged less than 30 years, the HPV OncoTect® E6 E7 mRNA Assay correctly identified 90% of women with either CIN2 or CIN3. It was not stated what happened longer-term to the women who had CIN2 or CIN3 that the HPV OncoTect® E6 E7 mRNA Assay had not picked up.

In a published Greek study, 189 biopsies were performed following screening of more than 4,000 cervical samples from women aged 21-65. The HPV OncoTect® E6 E7 mRNA Assay correctly identified women who were either normal or had only CIN1 in 74% of cases compared to 39% of cases tested using a high risk HPV test called CLART® HPV2 (from Genomica SAU, and not currently used in NHS screening).

In terms of further research, it would be useful to carry out long-term prospective clinical studies to find out how accurate the OncoTect® E6 E7 mRNA Assay is when used in a large representative sample of the UK screening population. A substantial study (using independent evaluation) would also be needed to compare the OncoTect® E6 E7 mRNA Assay directly against currently approved assays used for HPV triage testing for abnormal screening results before a referral to colposcopy is made, to find out whether it could reduce the number of false positives whilst not missing any more cases that do require colposcopy. A key research question would be does the OncoTect® E6 E7 mRNA Assay miss fewer cases of significant disease (i.e. requiring treatment) when used to triage low grade cytology compared to triaging with high risk HPV testing?
Cervical screening is a government priority area. Any changes to the NHS Cervical Screening Programme would need to be considered and approved by the Department of Health’s Advisory Committee on Cervical Screening. An expert has commented that because of the high number of colposcopies that turn out to be false alarms, a better way of triage testing before referral is needed. If the HPV OncoTect® E6 E7 mRNA Assay provides a more accurate way of identifying which women are at increased risk of developing cervical cancer than current methods, then the number of women needing to undergo colposcopy and biopsy could be reduced.

Cervical cancer is a clinical priority area, although following the introduction of national HPV vaccination in 2008 it may become less common in the future. A more accurate way of identifying women at increased risk of developing cervical cancer may mean that fewer women need to undergo invasive...
investigative procedures, which may improve quality of life and compliance. It could also lead to a reduction in demand for colposcopy and pathology services, which could potentially result in resource savings.

**Lay summary**

The HPV OncoTect® E6 E7 mRNA Assay is a new test for early signs of possible cancer of the cervix (neck of the womb). Some women who have a ‘smear’ test for cervical cancer may then be told that they need to have another test in hospital (a colposcopy) to check out an abnormal screening result. Usually the hospital test gives the ‘all clear’, but it can be a worrying and unpleasant time for the woman. This new test is designed to help decide which women really need to have this hospital test. Using it may mean that fewer women have to have a colposcopy. Some studies have suggested that it may be a useful test, but more studies are needed before we can be sure whether it would really help.

**Searches completed March 2012**

**References**


11. Coquillard G, Palao B and Patterson B. Quantification of intracellular HPV E6/E7 mRNA expression increases the specificity and positive predictive value of cervical cancer screening compared to HPV DNA. Gynecologic Oncology 2011;120:89-93.
