New Generation HPV Test Technologies

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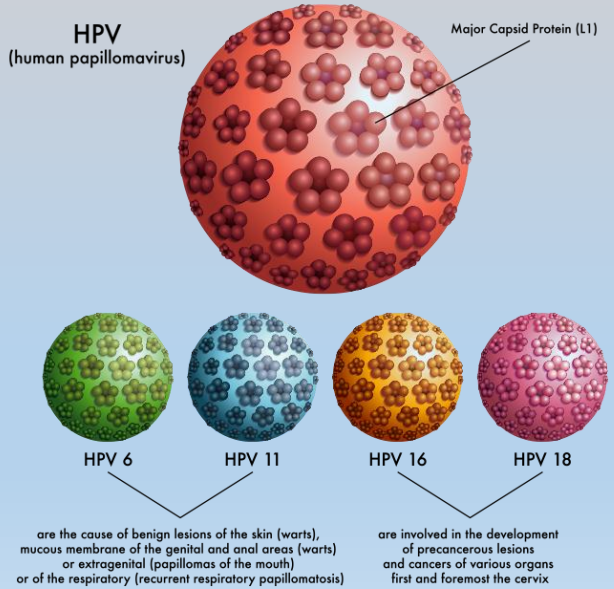
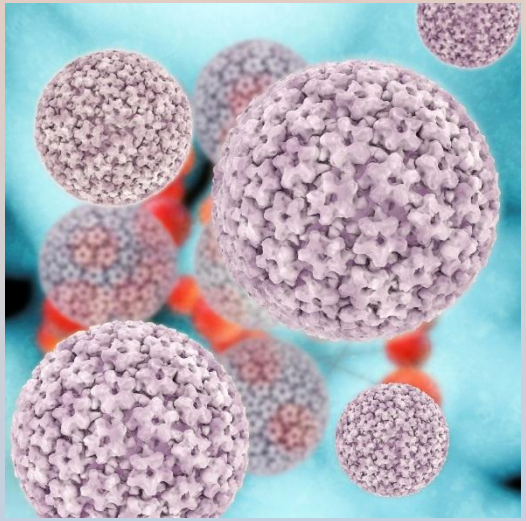


Introduction

- High-risk HPV is recognised as the single major cause of cervical cancer
- High-risk HPV DNA is found in 99.7% of cervical carcinomas
- The risk of developing cervical cancer increases by 250 fold for women with persistent high-risk HPV infection
- Without HPV infection cervical cancer is rare
- There are more than 100 commercially available HPV assays
- Detection of 13-14 high risk HPV genotypes including HPV16 and HPV18 allows for improved risk assessment and patient management



Introduction

- New technologies resulting from this increased understanding are changing our approach to cervical cancer prevention
- HPV test technologies will play a critical role in cervical screening in countries where HPV tests become the primary screening test
- Having robust technology will be critical for the continued success of cervical screening both through sensitive detection of women at risk of cervical cancer and by accurately excluding women who are at very low risk, with negative HPV test results
- These technologies are constantly evolving and will continue to evolve for future years

Nucleic Acid Amplification Assays

- Target amplification is the most flexible and sensitive of all HPV analysis techniques
- These assays can also be performed in multiplex, whereby multiple target sequences can be amplified simultaneously

1) **Real-time Polymerase Chain Reaction (PCR)**
   - Real-time PCR allows in-vitro multiplication of unique regions of DNA during a thermocycling procedure and detection of amplicons with fluorescently labelled probes (one target per fluorescence channel).
   - This technology can be used for detection, viral load quantitation, DNA sequencing, and mutation analysis
2) **Tagging Oligonucleotide Cleavage Extention (TOCE)** and **Dual-Priming-Oligonucleotide (DPO)**
   - This is a variation of real-time PCR for high-multiplex detection of DNA targets during a thermocycling procedure. Differentiation of amplicons is done via melt temperature analysis.
3) **Transcription-mediated amplification (TMA)**
   - TMA is used to amplify RNA targets in an isothermal procedure. The RNA amplicons are then detected by hybridisation with chemiluminescent labelled probes.

Nucleic Acid Amplification Assays

Three new generation HPV test technologies are:

Aptima HPV Assay – E6/E7 mRNA detection

Cepheid Genexpert – E6/E7 DNA amplification

Seegene Anyplex II HPV Detection – TOCE technology

All are Nucleic Acid Amplification Assays

All are used in Australia but not currently in New Zealand

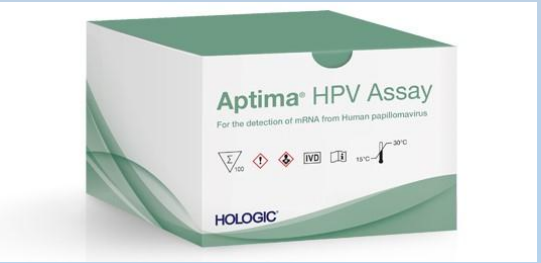
Aptima HPV Assay

- The Aptima HPV Assay is an In vitro nucleic acid amplification test for the qualitative detection of E6/E7 viral **messenger RNA** from 14 high risk types of HPV in cervical specimens
- The high risk HPV types detected by the assay include: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68.
- This test does not discriminate between the 14 high-risk subtypes, but can be run simultaneously with Aptima Genotyping Assay which identifies HPV 16, 18 & 45

Aptima® HPV Assay

Aptima HPV Assay

- The Aptima HPV assay involves 3 main steps which take place in a single tube:
  - Target capture
  - Target amplification by Transcription-Mediated Amplification
  - Detection of amplification products by the Hybridization Protection Assay
- The assay incorporates an internal control to monitor nucleic acid capture, amplification and detection; as well as operator or instrument error

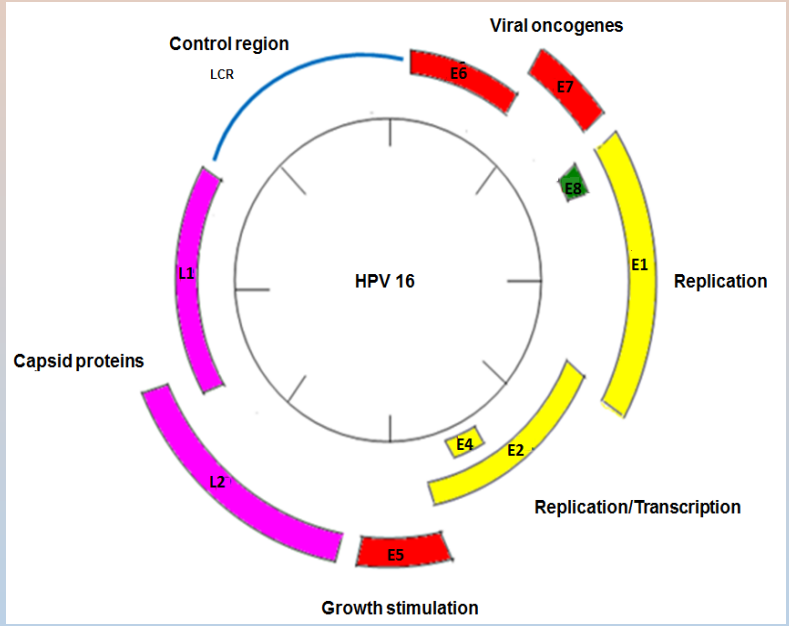


Aptima HPV Assay

- This assay has excellent sensitivity and specificity
- Same sensitivity as DNA-based tests for CIN 2+
- Higher specificity because an RNA-based test detects actively replicating virus whereas DNA-based tests detect the presence of viral DNA
- Has an internal process control
- The assay can be used with the Direct Tube Sampling System (DTS), the Tigris DTS System or the Panther System
- 250 tests can be carried out in approximately five hours.

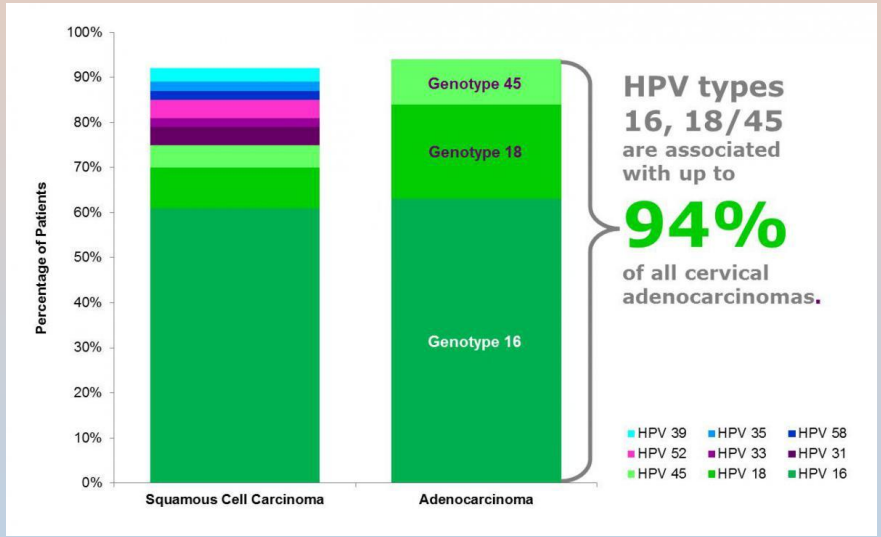
Aptima HPV Assay

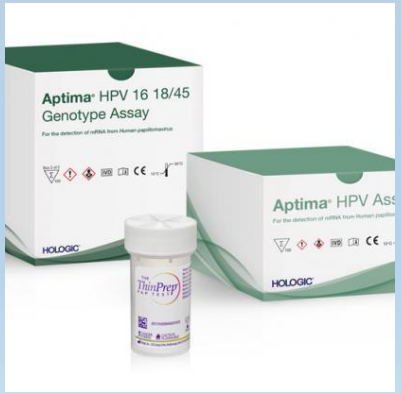
- **E6 & E7** controls cell cycle entry (all HPV types) which then allows genome amplification in the mid-layer of the epithelium
- Targeting E6/E7 mRNA identifies the presence of actively replicating high-risk HPV
- As disease progresses HPV DNA levels decrease whereas E6/E7 mRNA expression increases



Aptima HPV 16 18/45 Genotyping Assay

- HPV 16, 18 and 45 genotyping assay
- Targets HPV types that pose the largest risk to women
- Data suggests that up to 94% of all cervical adenocarcinomas are linked to HPV types 16, 18, and 45
- The Panther System runs the Aptima HPV and HPV genotype assays simultaneously
- <u>Limitations:</u>
  - The Aptima HPV 16 18/45 genotyping assay can differentiate HPV 16 from HPV18 and/or HPV 45, but does not differentiate between HPV 18 and HPV 45.





Cepheid Xpert HPV Assay

- The Xpert HPV assay is a qualitative in vitro test for the detection of the E6/E7 region of the viral DNA genome from high risk HPV in patient specimens
- Optimised detection of 14 high risk HPV subtypes reported as:
  - HPV16
  - HPV18/45
  - Other hrHPV (31, 33, 35, 32, 58, 51, 59, 39, 56, 66, 68)
- Targeting the E6/E7 oncogene eliminates concerns of L1 gene deletion
- The Xpert HPV assay is an automated test for qualitative detection and differentiation of HPV DNA

Cepheid Xpert HPV Assay

- The samples are processed as individual cartridges
- Different sized capacity machines with samples being processed one at a time up to 80 tests in one hour
- The GeneXpert System is available in a one, two, four, 16, 48, or 80-module configuration.
- On demand HPV testing!

Cepheid Xpert HPV Assay

- The assay is performed on Cepheid GeneXpert Instrument System
  - Automates and integrates sample processing, cell lysis, purification, nucleic acid amplification and detection of target sequences in clinical samples by using RT-PCR
- The system requires the use of single-use disposable GeneXpert cartridges that hold the PCR reagents, house the sample, and carry out the PCR processing
- Test results are reported for overall high-risk HPV status, as well as the presence of high-risk HPV genotypes

Cepheid Xpert HPV Assay

- A Sample Adequacy Control (SAC) and a Probe Check Control (PCC) are also included in the cartridge
- There are six colour channels containing primers and probes for the detection of specific genotypes or pooled results

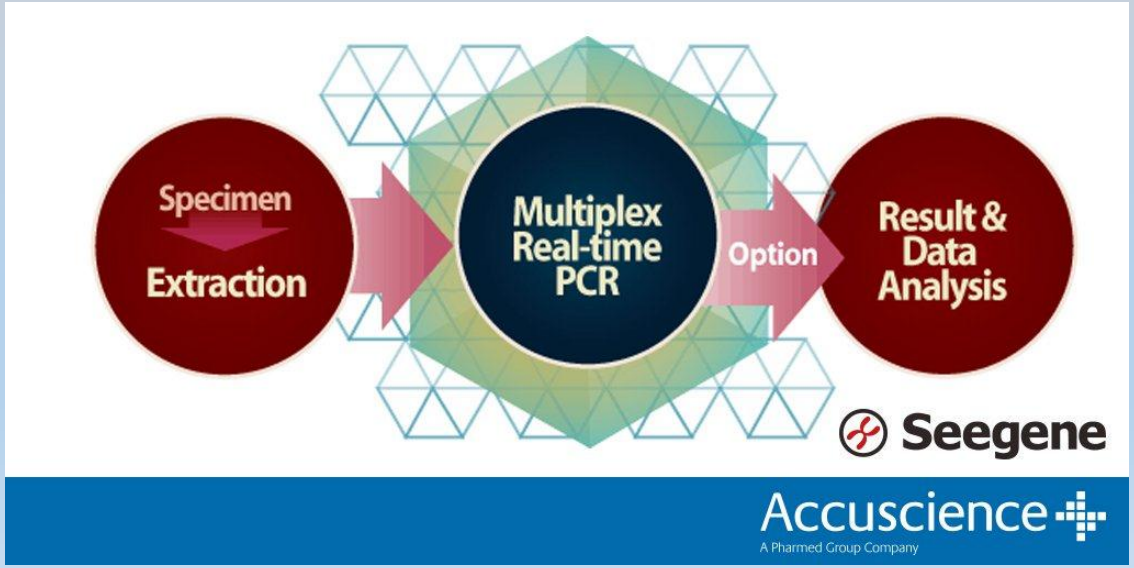
Seegene Anyplex II HPV Detection

- Seegene Anyplex II HPV detection is a new multiplex, real-time PCR assay
- Based on Seegene's proprietary DPO™ and TOCE™ technologies
- This assay is performed on a multiplex real-time PCR instrument and provides an accurate screening test of HPV infection in a single reaction
- The TOCE technology uses artificial, template-based melting temperature instead of the current, probe-based detection principle and distinguishes multiple targets in a single fluorescence channel in a real-time PCR reaction
- In addition, the cyclic-catcher melting temperature analysis with TOCE enables a semi-quantitative estimation of viral load through repeated melting temperature analysis expression during the TOCE reaction

Seegene Anyplex II HPV Detection

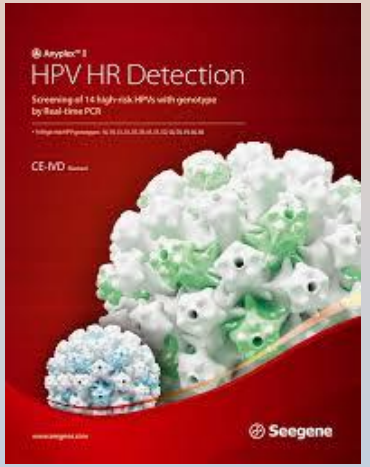
- HPV HR detection
  - 14 high-risk genotypes in a single reaction
- HPV 28 detection
  - 19 high-risk and 7 low-risk HPV's from a single real time PCR reaction

Both assays provide the individual HPV type-specific genotype for every HPV type detected



Seegene Anyplex II HPV HR Detection

- Simultaneous detection of 14 high-risk HPV genotypes in a single reaction
  - 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68
- Multiplex real-time PCR with high sensitivity and specificity by utilization of DPO and TOCE technologies
- Endogenous internal control for assay validity



Seegene Anyplex II HPV 28 Detection

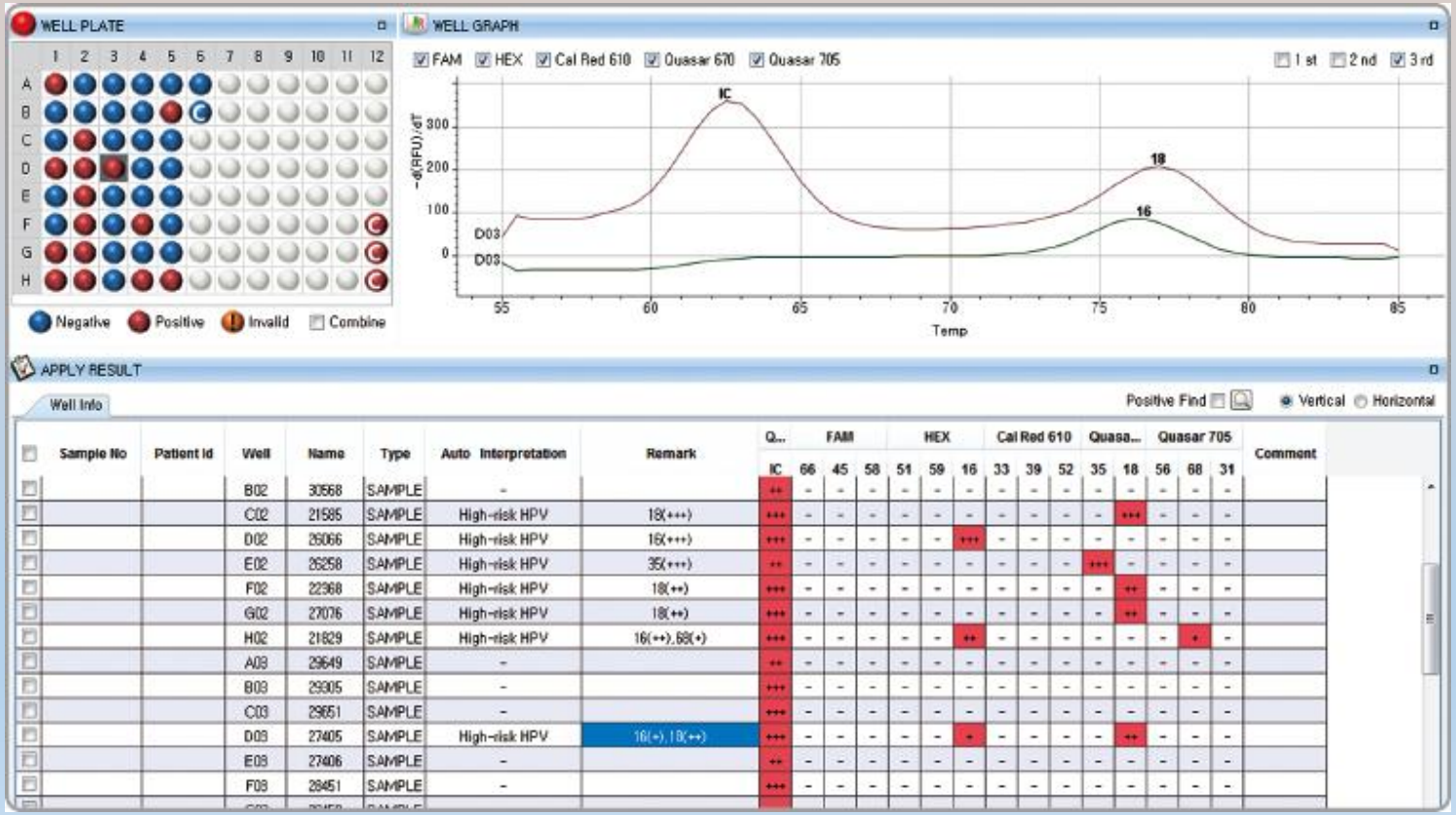
- Accurate genotyping of 28 HPV types in a single reaction
  - 19 high risk HPV genotypes: 16, 18, 26, 31, 33, 35, 39, 45, 51, 51, 53, 56, 59, 66, 68, 69, 73, 82
  - 9 low risk HPV genotypes : 6, 11, 40, 42, 43, 44, 54, 61, 70
- Multiplex real-time PCR with high sensitivity and specificity by utilization of DPO and TOCE technologies
- Endogenous whole process control for assay validity

Seegene Anyplex II HPV HR / 28 Detection

- Automated extraction and PCR setup
  - Microlab NIMBUS IVD
  - Microlab STARlet IVD
- Real-time PCR with the CFX96
- Convenient data interpretation by Seegene Viewer
- Run 184 samples in 7.5 hours

Seegene Anyplex II HPV HR / 28 Detection

- Quick and easy data analysis and interpretation



Concluding Comments

- This is a constantly evolving field of technology
- The use of HPV testing in cervical cancer prevention varies greatly between countries internationally, as does the population context within which screening is offered
- Different technologies offer different features which may be beneficial in different contexts
- In New Zealand, laboratories choose the HPV test technology they wish to use within requirements defined by the NCSP