HPV Detection: The Evolving Paradigm

Peter Lowe
AACB AIMS Combined Scientific Meeting
September 2016
HPV Biology and Lifecycle
HPV Types

201 HPV types isolated from humans

Classification based on sequence of 291 bp segment in L1

• Group 1 – carcinogenic
  • 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59

• Group 2A – Probably carcinogenic
  • 68

• Group 2B – Possibly carcinogenic
  • 26, 30, 34, 53, 66, 67, 69, 70, 73, 82, 85, 97

96% of cancers

2.6% of cancers

HPV Genome

Circular DNA Genome ~ 8000 bp

Early region – viral replication
- E1, E2, E4, and E5 required for viral replication
- E6 and E7 encode viral oncoproteins

Late region – viral capsid
- L1, L2 encode capsid proteins
- Involvement in initial infection

Upstream Regulatory Region
- Control transcription of the viral genome
HPV Infection – Productive Infection

Either high- or low-risk types and usually follows mechanical disruption

L1 and L2 facilitates binding, internalization and replication producing nuclear episomes

E1 and E2 proteins regulate replication

   E6 and E7 expression is minimal

Epithelial cells begin to differentiate and L1 and L2 are expressed

Release of virions by degeneration of exfoliated cells

HPV DNA can be detected inside and outside of host cells
HPV Infection – Transforming Infection

Same cell entry process

HPV DNA linearizes and integrates into host genome
   Random locations within human genome

E2 gene is disrupted and overexpression of E6 and E7 oncogenes occurs

E6 and E7 in HR-HPV is different to LR-HPV
   E6 binds tumor-suppressor protein p53
      p53 normally prevents damaged cells from reproducing
   E7 binds tumor-suppressor protein retinoblastoma (pRb)
      Binding of pRb causes uncontrolled cell proliferation
HPV Lifecycle
Viral Integration

Productive Infection
Initial HPV infection

Transforming Infection
HPV DNA integration

Episomal DNA

Host Chromosomal DNA

High-Risk Persistent Infections

Increased probability of progression to disease
HPV Nucleic Acid distribution in Cervical Pre-Cancer

Adapted from Doorbar. Clinical Science 2006. 110(5):525-41
HPV Clearance and Progression

- 20% of CIN3 progresses to cancer in 5 years
- 50% of CIN3 progresses to cancer in 30 years

Schiffman et al., JNCI 2011;
2 Molecular Assay Design
Considerations for HPV Assay Design

Choice of target

How to measure performance and how to validate performance

Study design is important!

- Prevalence of disease
- Choice of clinical endpoint: – CIN2+, CIN3+
- Choice of LBC
- Colposcopy methodology & Histology Interpretation – result review

“5% of biopsies read as CIN2+ are downgraded to <CIN2; conversely, 6% of biopsies reported as <CIN2 are upgraded to CIN2+ following pathology review”

HPV Integration

- HPV DNA must linearize to integrate into human DNA
- L1 region can be deleted when HPV DNA integration occurs
- HPV assays that only target the L1 region are at risk for false negative results\textsuperscript{1-3}

### HPV Nucleic Acid Amplification Tests

**TGA ARTG: 18/8/2016**

<table>
<thead>
<tr>
<th>Company</th>
<th>Product</th>
<th>Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qiagen</td>
<td>Hybrid Capture 2</td>
<td>Whole genome probe DNA</td>
</tr>
<tr>
<td>Roche</td>
<td>Cobas HPV</td>
<td>L1 DNA</td>
</tr>
<tr>
<td>Abbott</td>
<td>RealTime HPV</td>
<td>L1 DNA</td>
</tr>
<tr>
<td>Integrated Sciences</td>
<td>Seegene Anyplex™ II HPV</td>
<td>L1 DNA</td>
</tr>
<tr>
<td>Genera Biosystems</td>
<td>PapType</td>
<td>L1 DNA</td>
</tr>
<tr>
<td>Hologic</td>
<td>Cervista HPV</td>
<td>L1, E6, E7 DNA</td>
</tr>
<tr>
<td>Cepheid</td>
<td>Xpert HPV</td>
<td>E6, E7 DNA</td>
</tr>
<tr>
<td>Becton Dickinson</td>
<td>BD Onclarity</td>
<td>E6, E7 DNA</td>
</tr>
<tr>
<td>ESL Biosciences</td>
<td>EUROIMMUN EUROArray HPV Test</td>
<td>E6, E7 DNA</td>
</tr>
<tr>
<td>Hologic</td>
<td>Aptima HPV</td>
<td>E6, E7 mRNA</td>
</tr>
</tbody>
</table>
HPV High Sensitivity – Concerns

More is not necessarily better

HPV assays are more sensitive than cytology but less specific

HPV DNA can be detected outside of host cells

HPV can be detected in the environment¹

“HPV DNA was detected in 32 out of the 179 (17.9%) fomite samples”

“HPV contamination was 2.7 times more frequent in gynaecological private practices than in hospitals”

Samples: - black lamp, glove box, white lamp, colposcope handle and lubricant’s tube

¹ Gallay et al. Human papillomavirus (HPV) contamination of gynaecological equipment, Sex Transm Infect 2015; 92(1):19-23
Very High Sensitivity

Spanish Study – cobas 4800 HPV

- **Clinical positive samples = 14.8 to 2,754,229 cells**
  - Mean = 2000

- **Viral load = 158 to 40,738,028 copies**
  - Mean = 31,000 copies

- **Leucocyte LOD = 5 cells**

HPV – Clinical Specificity

Low specificity will mean unnecessary colposcopies

Options to improve specificity

- Use assay with high specificity (clinical and analytical)
- Use partial genotyping
  - ~70% SCC – types 16 and 18
  - ~12% ADC – type 45
  - Genotype distribution varies
- Use both
Standard Assay Validation – Meijer Criteria

The candidate test should have a clinical sensitivity for \( \geq \text{CIN2} \) not less than 90\% of the clinical sensitivity of the HC2 test (the main evidentiary standard test) in women of at least 30 years of age. This high sensitivity translates into a high negative predictive value and allows extending the screening interval for test negative women.

The candidate test should have a clinical specificity for \( \geq \text{CIN2} \) not less than 98\% of the clinical specificity of the HC2 test (the main evidentiary standard test) in women of at least 30 years of age.

The candidate test should display intra-laboratory reproducibility and inter-laboratory agreement with a lower confidence bound not less than 87\%. The HC2 and GP5+/6+ PCR tests revealed high inter-laboratory agreements of at least 92\%.
3
The Data
Lazarus Long - Robert A. Heinlein

“If it can’t be expressed in figures, it is not science; it is opinion. It has long been known that one horse can run faster than another — but which one? Differences are crucial.”

In our case

~ 145 HPV assays

All designed different, evaluated differently and marketed differently

Who do we believe?

Can we use the published data to decide on the definitive HPV assay?
All Assays are NOT Equal

“Some disagreement in comparisons between the three DNA assays and Aptima is therefore not surprising, yet the DNA assays showed more inter-assay disagreement than expected”

Rebolj et al. Disagreement between Human Papillomavirus Assays: An Unexpected Challenge for the Choice of an Assay in Primary Cervical Screening, PLoS ONE 2014; 9(1)
Further Analysis – by Cytology

All results - 651

Normal cytology - 558

Abnormal cytology - 93

Further Analysis – by Histology

The high level of concordance in detecting high-grade CIN points out that the choice of an HPV assay for screening will not so much affect the high-risk women who should be recommended for treatment to avoid progression to cervical cancer. The likelihood that they will be detected through screening is high with any of the herein evaluated assays, which is consistent with a relatively high clinical sensitivity for each assay.

On the other hand, false-positive screening tests, i.e. positive HPV test results with harmless infections that do not lead to high-grade CIN, represent clinically inconsequential noise.

Goal of Cervical Screening Program – Detection of HPV

To detect HPV that is associated with cellular changes predictive of progression to cancer

NOT

The detection of any HPV present

UTOPIA

High Sensitivity – HPV assay should not miss clinical disease

High NPV – patient has confidence in result / program

High Specificity – HPV assay should only detect clinical disease

High PPV – clinician has confidence in result / program
Low HPV Assay Specificity?

LBC contains 3 fractions

1. Free virus
2. Desquamating cells with new virus
3. Dividing cells with integrated DNA

HPV assay detects 1, 2 & 3
Cytology detects 2 & 3

Natural clearance of virus
Assay Specificity

Financial Impact – 2010 data

Potentially $1,250,800 **clinically inconsequential noise**

<table>
<thead>
<tr>
<th>Assay</th>
<th>Relative Specificity</th>
<th>Colposcopy Data @</th>
<th>Cost @</th>
<th>Additional Colposcopies</th>
<th>Additional Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytology</td>
<td>1.00</td>
<td>70900</td>
<td>$21,200,000</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hybrid Capture 2</td>
<td>0.85</td>
<td>81251</td>
<td>$24,295,200</td>
<td>10351</td>
<td>$3,095,200</td>
</tr>
<tr>
<td>Cobas HPV</td>
<td>0.85</td>
<td>81890</td>
<td>$24,486,000</td>
<td>10990</td>
<td>$3,286,000</td>
</tr>
<tr>
<td>RealTime HPV</td>
<td>0.87</td>
<td>79975</td>
<td>$23,913,600</td>
<td>9075</td>
<td>$2,713,600</td>
</tr>
<tr>
<td>Seegene Anyplex™ II HPV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PapType</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cervista HPV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xpert HPV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EUROIMMUN EUROArray HPV Test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aptima HPV</td>
<td>0.90</td>
<td>77848</td>
<td>$23,277,600</td>
<td>6948</td>
<td>$2,077,600</td>
</tr>
</tbody>
</table>


@ Expenditure and resource utilisation for cervical screening in Australia, Lew et al, BMC Health Services Research (2012) 12, 446

Patient Impact – Emotional, financial

New NCSP has been modeled with the aim providing cost-benefit outcomes
Longitudinal Data

![Graph showing incidence of CIN3+ over time](image)

**Fig 3** Kaplan-Meier plots of cumulative incidence rate for CIN3+ for women according to baseline test results in first 72 months of follow-up, excluding Denmark and Tübingen

Longitudinal Data

Fig 2 | Kaplan-Meier plots of cumulative incidence rate for CIN3+ for women according to baseline test results in first 72 months of follow-up, excluding Denmark and Tübingen

Dillner et al. Long term predictive values of cytology and human papillomavirus testing in cervical cancer screening: joint European cohort study BMJ 2008;377:a1754
Longitudinal Studies

Kaiser – 5 years +
  HC2
Roche – ATHENA – 3 years
  HC2 & cobas
Hologic – CLEAR – 3 years
  HC2 & Aptima
Hologic – GAST – 5-6 years
  HC2 & Aptima
Recent Longitudinal Data
GAST – 5-6 year

<table>
<thead>
<tr>
<th></th>
<th>HC2 at baseline</th>
<th></th>
<th>Aptima at baseline</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>95% CI</td>
<td>%</td>
<td>95% CI</td>
</tr>
<tr>
<td>NPV</td>
<td>99.9</td>
<td>99.8 to 100</td>
<td>99.1</td>
<td>(98.8-99.4)</td>
</tr>
<tr>
<td>PPV</td>
<td>20.0</td>
<td>14.9 to 26.1</td>
<td>23.5</td>
<td>17.2 to 29.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15.3 to 33.5</td>
<td>24.4</td>
<td>(15.3 to 33.5)</td>
</tr>
<tr>
<td>Adjusted sensitivity for CIN2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline and follow up</td>
<td>93.5 to 100</td>
<td>85.8</td>
<td>75.2 to 97.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>37.2% (26.9-51.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted sensitivity for CIN2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline and follow up</td>
<td>95.0</td>
<td>94.9 to 95.1</td>
<td>96.2</td>
<td>96.0 to 96.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>98.4 (98.1 to 98.7)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1.2% difference in specificity = 23% less referrals

Only the difference in specificity of HC2 vs Aptima is significant, all the other values are not

Iftner et al. ICC Japan 2016
Molecular Testing for HPV

The choice is easy – isn’t it?
Clear?
Thank you
Selection of Target

“The advantage of targeting the E1 or L1 region is that this has the potential to detect many, perhaps all, HPV types.

The major disadvantage is that this has the potential not to occur if the virus is present in an exclusively integrated form, as is indeed the case for many advanced abnormalities.

Thus, the target for PCR can be absent for regions other than E6 and E7. On the other hand, targeting the E6 or E7 regions will detect all samples infected with high-risk HPV, provided that primers for all relevant HPV types are used.”

### Athena Data

Modelling for the NCSP predicts 1.3 million HPV tests / year

<table>
<thead>
<tr>
<th>Contributing factor</th>
<th>Rate per 100,000 of missed CIN2+</th>
<th>Rate per year of missed CIN2+</th>
<th>Women at risk of missed disease diagnosis per year# ^</th>
</tr>
</thead>
<tbody>
<tr>
<td>No specimen collected</td>
<td>5.4</td>
<td>70</td>
<td>18</td>
</tr>
<tr>
<td>Sensitivity of HPV assays - Clinician-collected samples</td>
<td>556</td>
<td>7,228</td>
<td>1,908</td>
</tr>
<tr>
<td>Sensitivity of HPV assays - Self-collected samples</td>
<td>780</td>
<td>10,140</td>
<td>2,677</td>
</tr>
</tbody>
</table>

# estimated 1.3 million HPV tests per year in Australia

^ Assume 66% of all CIN2 regress within 3 years

<table>
<thead>
<tr>
<th>Contributing factor</th>
<th>Rate per 100,000 of missed CIN3+</th>
<th>Rate per year of missed CIN3+</th>
</tr>
</thead>
<tbody>
<tr>
<td>No specimen collected</td>
<td>2.9</td>
<td>38</td>
</tr>
<tr>
<td>Sensitivity of HPV assays - Clinician-collected samples</td>
<td>234</td>
<td>3,042</td>
</tr>
<tr>
<td>Sensitivity of HPV assays - Self-collected samples</td>
<td>368</td>
<td>4,784</td>
</tr>
</tbody>
</table>

* estimated 1.3 million HPV tests per year in Australia