Recent Advances in HPV Testing

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Conflict of Interest Disclosures

- Gen-Probe: Scientific Advisory Board, Research Funding, Clinical Trials
- bioMerieux: Scientific Advisory Board, Research Funding, Clinical Trials
- Hologic: Research Funding
- Abbott: Speaker’s Bureau
Learning Objectives

At the completion of this session the attendee will

- Explain the basic steps in the pathogenesis of HPV related cervical disease
- State the roles of HPV molecular testing and High-Risk (HR) HPV genotyping in both the prevention, detection and monitoring of cervical disease
- Describe the proposed clinical differences between the detection of HR HPV DNA and HR HPV mRNA
- List the optimal parameters of a nucleic acid based HR HPV diagnostic test
Background - Virus

- *Papovaviridae* family
- Non-enveloped, icosahedral capsule
- Double stranded, circular DNA, 7,900 bp
- 10 viral proteins, eight (8) early gene products and two (2) late gene products
  - The early gene products are involved in replication (active infection) and oncogenesis
  - Included in the viral DNA are 2 oncogenes (E-6, E-7) and a protein that suppresses expression of the oncogenes (E-2).
  - The late gene products encode structural proteins for the viral capsid
- 200 different genetic types
Disease States and HPV Types

- Plantar warts
- Common warts
- Flat warts
- Cutaneous lesions
- Epidermodysplasia verruciformis
- Respiratory papillomatosis
- Focal epithelial hyperplasia of Heck
- HNSCC and OPSCC
- Conjunctival papillomas and carcinomas
- Condyloma acuminata
- Cervical intraepithelial neoplasia
  - Low risk
  - High risk
- Cervical carcinoma

1, 2, 4, 63
1, 2, 7, 4, 26, 27, 29, 41, 57, 65, 77, 1, 3, 4, 10, 28
3, 10, 26, 27, 28, 38, 41, 49, 75, 76
6, 11, 16, 30, 33, 36, 37, 38, 41, 48, 60, 72, 73
2, 3, 10, 5, 8, 9, 12, 14, 15, 17, 19-25, 36-38, 47, 50
6, 11
13, 32
16+
6, 11, 16
6, 11, 30, 42, 43, 45, 51, 54, 55, 70
6, 11, 42, 43, 44
16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68
16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68
HPV Genital Infection Outcomes

Genital warts
LR HPV 6, 11

Colposcopy: mosaic tile pattern
Biopsy: koilocytosis with mild dysplasia (CIN 1)

Colposcopy: Diffuse white patch
Pap: Severe dysplasia/SCC in situ (CIN 3)
HPV Viral Integration

Initial HPV infection
- Episomal DNA
- Host Chromosomal DNA

HPV DNA integration
- High-Risk Infections

• Generally low levels of E6/E7 mRNA expression

• Increased E6/E7 mRNA expression
• Increased probability of progression to disease
HPV E6 oncoprotein activates p53, which in turn induces apoptosis. DNA damage results in G1 arrest and the expression of p21, which inhibits cell cycle progression. HPV E7 oncoprotein disrupts the complex between pRB and E2F1, preventing the cell from entering the S phase. Cyclins and cyclin-dependent kinases (CDKs) are essential for cell cycle progression, and their dysregulation is a hallmark of HPV infections. DNA damage can also lead to rapid degradation of p53 by a cellular ubiquitin ligase, further facilitating oncogenic processes. 

Burd, EM. Clin Micro Rev. 16:1. 2003
Comparison of Three Management Strategies for Patients With Atypical Squamous Cells of Undetermined Significance: Baseline Results From a Randomized Trial

Diane Solomon, Mark Schiffman, Robert Tarone

**ASC-US**
- Immediate colposcopy
- HPV and thin-layer cytology
  - HPV+ or HSIL Triage
  - colposcopy
- Cytology alone
  - HSIL+ Triage
  - colposcopy
## Study Data

<table>
<thead>
<tr>
<th>CIN3+</th>
<th>% Sensitivity</th>
<th>% Referral</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC II</td>
<td>96.3</td>
<td>56.1</td>
<td>10.0</td>
<td>99.5</td>
</tr>
<tr>
<td>HSIL + Cy</td>
<td>44.1</td>
<td>6.9</td>
<td>37.5</td>
<td>96.5</td>
</tr>
<tr>
<td>LSIL + Cy</td>
<td>64.0</td>
<td>26.2</td>
<td>14.3</td>
<td>97.1</td>
</tr>
<tr>
<td>ASCUS + Cy</td>
<td>85.3</td>
<td>58.6</td>
<td>8.5</td>
<td>97.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CIN2+</th>
<th>% Sensitivity</th>
<th>% Referral</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC II</td>
<td>95.9</td>
<td>56.1</td>
<td>19.6</td>
<td>98.9</td>
</tr>
<tr>
<td>HSIL + Cy</td>
<td>34.8</td>
<td>6.9</td>
<td>58.1</td>
<td>92.0</td>
</tr>
<tr>
<td>LSIL + Cy</td>
<td>59.2</td>
<td>26.2</td>
<td>25.9</td>
<td>93.6</td>
</tr>
<tr>
<td>ASCUS + Cy</td>
<td>85.0</td>
<td>58.6</td>
<td>16.7</td>
<td>95.8</td>
</tr>
</tbody>
</table>

Solomon et al. JNCI. 93:293-299
Sensitivity of HPV+ for Detection of HSIL

Cohort analysis of 5,671 women age >30

## Performance of HC II and Cervical Cytology in Women Aged 30 Years or More in Cross Sectional Studies

<table>
<thead>
<tr>
<th>Population</th>
<th>No.</th>
<th>CIN2+ (%)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>NPV Combo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pap</td>
<td>HPV</td>
<td>Pap + HPV</td>
</tr>
<tr>
<td>Germany</td>
<td>7,592</td>
<td>1.01</td>
<td>33.8</td>
<td>85.7</td>
<td>93.5</td>
</tr>
<tr>
<td>UK</td>
<td>10,358</td>
<td>0.90</td>
<td>72.2</td>
<td>96.9</td>
<td>100</td>
</tr>
<tr>
<td>Mexico</td>
<td>6,115</td>
<td>1.41</td>
<td>57.0</td>
<td>94.2</td>
<td>97.7</td>
</tr>
<tr>
<td>Costa Rica</td>
<td>6,176</td>
<td>1.75</td>
<td>80.4</td>
<td>86.3</td>
<td>92.2</td>
</tr>
<tr>
<td>South Africa</td>
<td>2,925</td>
<td>3.56</td>
<td>74.0</td>
<td>84.9</td>
<td>87.0</td>
</tr>
<tr>
<td>China</td>
<td>1,936</td>
<td>4.34</td>
<td>94.0</td>
<td>97.6</td>
<td>100</td>
</tr>
<tr>
<td>Baltimore, MD</td>
<td>1,040</td>
<td>0.48</td>
<td>60.0</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

CIN2+ = CIN 2, 3 or cancer

Wright et al. 2003. ACOG. 103:304
# 2010 Indications for HPV Testing

<table>
<thead>
<tr>
<th>Indication</th>
<th>Age Restriction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Routine cervical cancer screening in conjunction with cervical cytology (dual testing or cotesting)</td>
<td>30 years and older</td>
</tr>
<tr>
<td>Initial triage management of women with a cytologic result of atypical squamous cells of undetermined significance (ASC-US)</td>
<td>21 years and older</td>
</tr>
<tr>
<td>Initial triage management of postmenopausal women with cytologic result of low-grade squamous intraepithelial lesion (LSIL)</td>
<td>Postmenopausal</td>
</tr>
<tr>
<td>Postcolposcopy management of women of any age with initial cytologic result of atypical glandular cells (AGCs) or atypical squamous cells, cannot exclude a high-grade squamous intraepithelial lesion (ASC-H) (when initial workup does not identify a high-grade lesion)</td>
<td>None</td>
</tr>
<tr>
<td>Postcolposcopy management of women 21 years and older with initial cytologic results of ASC-US or LSIL (when initial colposcopy does not identify a high-grade lesion)</td>
<td>21 years and older</td>
</tr>
<tr>
<td>Posttreatment surveillance</td>
<td>None</td>
</tr>
</tbody>
</table>
2011 ACS-ASCCP-ASCP Draft Guidelines
Benefit versus Harm

• Frequency of screening:
  • Women (average risk for cancer) 21-29 and women ≥30 with 2 or more negative cytology: 3 years for cytology alone

• Screening Strategies for Women 30 and Older
  • Cotesting with cytology and HPV testing be used for general population screening in preference to one test alone
  • If both tests are negative, rescreening should be done at 3-5 year intervals
  • General population screening should not be done at intervals shorter than 3 years regardless of the screening modality employed
Use of HPV Genotyping to Manage HPV HR* Positive / Cytology Negative
Women 30 Years and Older

HPV HR Positive / Cytology Negative

- HPV 16/18 (+)
  - Repeat BOTH cytology and HR HPV test @ 12 months
  - Both negative
    - Colposcopy
  - Cytology negative HPV (+)
    - Routine screening @ 2 years
  - Cytology abnormal any HPV result
    - Colposcopy

- HPV 16/18 (-)
  - Manage per ASCCP Guideline

* Test that detects any of the 14 high-risk (oncogenic) types of HPV
# Relative Risk of High Grade Disease by Genotype – Athena Study

<table>
<thead>
<tr>
<th>HPV Infection Type</th>
<th>Relative Risk of &gt;CIN2</th>
<th>Relative Risk of &gt;CIN3</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV-16+ vs HPV-</td>
<td>42.0 (20.1-87.5)</td>
<td>70.9 (21.8-231.1)</td>
</tr>
<tr>
<td>HPV-16+/HPV18+ vs HPV-</td>
<td>32.5 (15.5-67.9)</td>
<td>56.4 (17.3-183.6)</td>
</tr>
<tr>
<td>HR-HPV+ vs HPV-</td>
<td>18.6 (9.0-38.4)</td>
<td>29.7 (9.3-95.2)</td>
</tr>
<tr>
<td>12 other HPV+ vs HPV-</td>
<td>11.4 (5.3-24.7)</td>
<td>15.7 (4.6-54.0)</td>
</tr>
<tr>
<td>HPV-18+ vs HPV-</td>
<td>5.8 (1.3-26.5)</td>
<td>15.4 (2.6-90.1)</td>
</tr>
<tr>
<td>HPV-16+ vs 12 other HPV+</td>
<td>3.7 (2.4-5.7)</td>
<td>4.5 (2.5-8.2)</td>
</tr>
</tbody>
</table>

Characteristics of the Ideal HPV Test

- **High clinical sensitivity** ⇒ detect high-risk HPV infections that lead to cervical disease
  - High negative predictive value for disease (>99.5%)

- **High clinical specificity** ⇒ do not detect transient high-risk HPV infections that will not lead to cervical disease
  - High positive predictive value for disease
HPV Molecular Detection Methods

- HR HPV DNA detection
- HR HPV DNA genotyping
- HR HPV DNA quantification
- HR E6/E7 mRNA detection
- HR E6/E7 mRNA genotyping
- HR E6/E7 mRNA quantification
- Direct sequencing
- Clonal sequencing
- Integration assays (RO)
HPV Molecular Detection Methods

- HR HPV DNA detection
- HR HPV DNA genotyping
- HR HPV DNA quantification
- HR E6/E7 mRNA detection
- HR E6/E7 mRNA genotyping
- HR E6/E7 mRNA quantification
- Direct sequencing
- Clonal sequencing
- Integration assays (RO)
<table>
<thead>
<tr>
<th>Test</th>
<th>Method</th>
<th>Specimen</th>
<th>HPV Targets</th>
<th>IC</th>
<th>Genotyping</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digene Hybrid Capture 2 (Qiagen)*</td>
<td>Hybrid Capture Rapid Capture System</td>
<td>STM</td>
<td>16,18,31,33,35,39 45,51,52,56,58,59,68</td>
<td>No</td>
<td>No Future reflex</td>
</tr>
<tr>
<td>Cervista HPV HR Test (Hologic)* Day SP JCV:45:S63</td>
<td>Invader Chemistry</td>
<td>PreservCyt: 1 ml</td>
<td>16,18,31,33,35,39,45 51,52,56,58,59,66,68</td>
<td>Human histone 2 gene</td>
<td>Reflex to HPV 16, 18 Assay</td>
</tr>
<tr>
<td>cobas® 4800 HPV Test System*</td>
<td>Real Time PCR cobas 4800</td>
<td>Cobas collection PreserCyt Surepath</td>
<td>16, 18, 31, 33, 35, 39 45, 51, 52, 56, 58, 59, 66, 68</td>
<td>β-globin</td>
<td>Yes HPV 16, 18</td>
</tr>
<tr>
<td>Roche Amplicor HPV Test</td>
<td>PCR</td>
<td>PreserCyt Surepath</td>
<td>16,18,31,33,35,39,45 51 52, 56, 58, 59, 68</td>
<td>β-globin</td>
<td>No</td>
</tr>
<tr>
<td>NextGen (Qiagen) Eder P. JCV:45:S85</td>
<td>Modified Hybrid Capture JE2000</td>
<td>STM: 50 µl</td>
<td>16,18,31,33,35,39,45 51,52,56,58,59,66,68 82</td>
<td>No</td>
<td>No Future reflex</td>
</tr>
<tr>
<td>RealTime HPV Assay (Abbott) Huang S. JCV:45:S13</td>
<td>Real Time PCR m2000sp m2000rt</td>
<td>PreservCyt: 0.4-.6 ml</td>
<td>16,18,31,33,35,39,45 51,52,56,58,59,66,68</td>
<td>β-globin</td>
<td>Yes HPV 16, 18</td>
</tr>
</tbody>
</table>

*IVD
<table>
<thead>
<tr>
<th>Test</th>
<th>LOD</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digene Hybrid Capture 2 (Qiagen)*</td>
<td>5,000 cps/rxn</td>
<td>Cross reactivity with 6,11,53,66,67,70,82/82v</td>
</tr>
<tr>
<td>Castle P JCM:46:2595</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cervista HPV HR Test (Hologic)*</td>
<td>**1250-2500 cps/rxn: 16,18,31,45,52,56</td>
<td>Clinical samples: 91.4% agreement with PCR/seq</td>
</tr>
<tr>
<td>Day SP JCV:45:S63</td>
<td>2,500-5,000 cps/rxn: 33,39,51,58,59,66,68</td>
<td>Neg: 1,6,11,42,43,44,53 Pos: 67:&gt;10³, 70:&gt;10⁴</td>
</tr>
<tr>
<td></td>
<td>5,000-7500 cps/rxn: 35</td>
<td></td>
</tr>
<tr>
<td>Cobas 4800 HPV Test (Roche)*</td>
<td>150 cps/ml: 45; 300 cps/ml: 31,33,39,51,59</td>
<td>100%: 25 LR types at &gt;10⁶</td>
</tr>
<tr>
<td>Package insert</td>
<td>600 cps/ml: 16,18,35,58</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1200 cps/ml: 56,66,65; 2400 cps/ml: 52</td>
<td></td>
</tr>
<tr>
<td>NextGen (Qiagen)</td>
<td>**&lt;1,000 cps/rxn to 1,700 cps/rxn</td>
<td>100%: 13 LR Types at 10⁷</td>
</tr>
<tr>
<td>Eder P. JCV:45:S85</td>
<td>Std cut off: 1,146-9,250 cps/rxn</td>
<td>Pos correlation with HC2+ 98.5%</td>
</tr>
<tr>
<td>RealTime HPV Assay (Abbott)</td>
<td>**500 cps/rxn: 16,18,35,39,45,51,59,66,68</td>
<td>14 HR-HPV: 100%</td>
</tr>
<tr>
<td>Huang S. JCV:45:S13</td>
<td>**2,000 cps/rxn: 31,33,52,56</td>
<td>15 LR-HPV: 100%</td>
</tr>
<tr>
<td></td>
<td>5,000 cps/rxn: 58</td>
<td></td>
</tr>
</tbody>
</table>

**Does increased sensitivity equate with better disease detection or more transient positives?**

*IVD
DNA Based Testing Methods
Qiagen Hybrid Capture® 2 HPV DNA Test

1. Release and denature nucleic acids
2. Hybridize RNA probe with target DNA
3. Capture RNA:DNA hybrids onto a solid phase
4. React captured hybrids with multiple antibody conjugates
5. Detect amplified chemiluminescent signal
Qiagen Rapid Capture System

Detects HR types: 16,18,31,33,35,39,45,51,52,56,58,59,68
## Table 8
Kaiser Study Data
hc2 High-Risk HPV DNA Test Performance versus Consensus Histology Results (CIN 2-3) Age-Specific Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Age &lt; 30</th>
<th>Age 30 - 39</th>
<th>Age &gt;39</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>287</td>
<td>233</td>
<td>365</td>
</tr>
<tr>
<td>Prevalence of Disease (%)</td>
<td>12.2</td>
<td>11.2</td>
<td>2.7</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>100.00</td>
<td>88.46</td>
<td>80.00</td>
</tr>
<tr>
<td>(35/35)</td>
<td>(23/26)</td>
<td>(8/10)</td>
<td></td>
</tr>
<tr>
<td>95% Confidence Interval</td>
<td>90.0-100</td>
<td>69.9-97.6</td>
<td>44.4-97.5</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>31.4</td>
<td>66.2</td>
<td>79.15</td>
</tr>
<tr>
<td>(79/252)</td>
<td>(137/207)</td>
<td>(281/355)</td>
<td></td>
</tr>
<tr>
<td>95% Confidence Interval</td>
<td>25.7-37.5</td>
<td>59.3-72.6</td>
<td>74.6-83.3</td>
</tr>
<tr>
<td>Negative Predictive Value (%)</td>
<td>100</td>
<td>97.86</td>
<td>99.29</td>
</tr>
<tr>
<td>(79/79)</td>
<td>(137/140)</td>
<td>(281/283)</td>
<td></td>
</tr>
<tr>
<td>Positive Predictive Value (%)</td>
<td>16.83</td>
<td>24.73</td>
<td>9.76</td>
</tr>
<tr>
<td>(35/208)</td>
<td>(23/93)</td>
<td>(8/82)</td>
<td></td>
</tr>
</tbody>
</table>
Qiagen Second Generation HPV Assays

High/Ultra-High and Low/Medium Systems

**QIAensemble™ SP / 2000**
- Sample Prep
- Analytical

**QIAconductor™ Software**

**QIAsymphony™ SP/QIAensemble™ 400**

- LBC Vials
- Urine Vials

- Co-collected DCM™ - HPV Urethral Swabs – CT/GC

**High/ultra-high throughput Instrument**
1-2000 tests/shift
floor model configuration

**Low/medium throughput instrument**
1-600 tests/shift
benchtop configuration

North Shore LIJ
Hologic Cervista HPV Invader Assay

1. Remove 1 ml of cervical specimen

2. Extract DNA

3. Add 10 ul of each DNA sample into 3 wells.

4. Denature 5 min at 95 °C

5. Prepare HPV Invader Reaction mixes

<table>
<thead>
<tr>
<th>Family</th>
<th>HPV Types</th>
</tr>
</thead>
<tbody>
<tr>
<td>A5/A6</td>
<td>51, 56, 66, HIST2H2BE</td>
</tr>
<tr>
<td>A7</td>
<td>18, 39, 45, 59, 68, HIST2H2BE</td>
</tr>
<tr>
<td>A9</td>
<td>16, 31, 33, 35, 52, 58, HIST2H2BE</td>
</tr>
</tbody>
</table>

6. Add 10 ul of reaction mix to the appropriate plate sector.

At 63 °C

7. Denature 5 min at 95 °C

8. Read signal in fluorescence plate reader

A5/A6  A7     A9
Invader® Technology

1a. HPV oligos form invasive structure on HPV DNA.
1b. HIST2H2BE oligos form invasive structure on genomic DNA.

2. Cleavase® enzyme recognizes structure and cleaves probe oligos.

3a. Flaps from HPV probe oligos form invasive structure on FAM FRET oligos.
3b. Flaps from HIST2H2BE probe oligos form invasive structure on Red FRET oligos.

4. Cleavase® enzyme recognizes structure and releases fluorophores from FRET oligos, creating fluorescence signal.

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Hologic Cervista HPV Automation

- **Processing Capabilities**
  - Up to 96 samples in ~2 hrs
  - 2 discrete batches of 48
  - Aliquot volumes from 0.5 ml to 2.0 ml

- **Instrument Platform:**
  - Tecan EVO® 150

- **Automated Steps:**
  - DNA extraction

- **Processing Capabilities:**
  - 48 or 96 samples per batch
### Table 11: Clinical Performance Summary of the Cervista™ HPV HR Test as Compared to Colposcopy/Central Histology Results (≥ CIN2) among Women with ASC-US Cytology

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Disease Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>92.8% (64/69)</td>
<td>44.2% (558/1263)</td>
<td>8.3% (64/769)</td>
<td>99.1% (553/563)</td>
<td>5.2% (69/1332)</td>
</tr>
<tr>
<td>95% CI:</td>
<td>(84.1% - 96.9%)</td>
<td>(41.5% - 46.9%)</td>
<td>(7.6% - 8.9%)</td>
<td>(98.1% - 99.6%)</td>
<td></td>
</tr>
</tbody>
</table>

Note: Among women with ASC-US cytology, there were 1.1% (15 out 1347) Cervista™ HPV HR indeterminate results with 95% CI: 0.7% to 1.8%.

### Table 12: Clinical Performance Summary of the Cervista™ HPV HR Test as Compared to Colposcopy/Central Histology Results (≥ CIN3) among Women with ASC-US Cytology

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Disease Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100% (22/22)</td>
<td>43% (563/1310)</td>
<td>2.9% (22/769)</td>
<td>100% (563/563)</td>
<td>1.7% (22/1332)</td>
</tr>
<tr>
<td>95% CI:</td>
<td>(85.1% - 100%)</td>
<td>(40.3% - 45.7%)</td>
<td>(2.4% - 3.0%)</td>
<td>(99.4% - 100%)</td>
<td></td>
</tr>
</tbody>
</table>

Note: Among women with ASC-US cytology, there were 1.1% (15 out 1347) Cervista™ HPV HR indeterminate results with 95% CI: 0.7% to 1.8%. CIN2 histology results (47) are considered negative for disease (≥ CIN3) in this table.
CIN3+

- Population based-cross sectional study
- 8,556 women aged 25-59 yr
- Overall rate for ASC-US or worse: 12.1 %
- Overall rate for CIN2+: 2.7%
- Overall rate for CIN3+: 1.6%
- Overall HPV+ rate:
  - 13.6% (HC2) vs 11.1% (C: Cervista) \( P < .000001 \)
- HPV rate with normal cytology
  - 7.9 % (HC2) vs 6.0% (C) \( P < .000001 \)
- HPV rate with ASC-US cytology
  - 41.4 % (HC2) vs 34.0% (C) \( P < .000001 \)

- ASC-US HR HPV rates: 59.4% (HC2) vs 48.5% (C)
- 30 yr +: cyto:WNL rates: 5.5% (HC2) vs 5.8% (C)
Roche Cobas 4800 HPV Test

- The Roche Diagnostics AMPLICOR HPV Test
  - PCR to target and amplify HPV DNA from 13 high-risk genotypes
  - Requires 250 ml of liquid cytology screening
  - Concurrent isolation and amplification of the β-globin gene assesses cellular adequacy and inhibition for each specimen
  - PreservCyt® (Hologic) or SurePath® (BD)

- The cobas® 4800 HPV Test System
  - PCR test specifically identifies HPV 16 and HPV 18 while concurrently detecting the rest of the HR types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) at clinically relevant infection levels
  - Cervical cells collected in cobas® PCR Cell Collection Media (Roche Molecular Systems, Inc.), PreservCyt® Solution (Cytyc Corp.) and SurePath® Preservative Fluid (BD Diagnostics-TriPath)
High-Risk HPV Testing in Women with ASC-US Cytology: ATHENA Study

47,208 women 21 yr+ with ASC-US cytology
16/18 genotyping: 1,923 pts: 4.1%

Abbott RealTime HPV Assay

- CE marked: Europe only
- Detects HR types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68
  Specifically identifies types 16/18
- Real-time PCR with GP5+/6+ primers
- Internal control: $\beta$-globin
PCR versus HC2
Abbott RealTime: Clinical Studies

CIN2+

CIN3+

Huang S. 2009 JCV:45:S19: 702 pts with abnormal cytology
## Abbott RealTime HPV Test: Clinical Studies

### Poljak M. 2009 Acta Derm APA 18:94: 95 w/CC 267 w/CIN3

<table>
<thead>
<tr>
<th>Test</th>
<th>Disease</th>
<th>Clinical Sensitivity</th>
<th>Analytical Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott</td>
<td>CIN3</td>
<td>91.8%</td>
<td>96.4%</td>
</tr>
<tr>
<td>HC2</td>
<td>Agree: 93.6%</td>
<td>89.1%</td>
<td>92.5%</td>
</tr>
<tr>
<td>Abbott</td>
<td>CC</td>
<td>88.4%</td>
<td>98.8%</td>
</tr>
<tr>
<td>HC2</td>
<td>Agree: 94.7%</td>
<td>87.4%</td>
<td>95.3%</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Test</th>
<th>Disease</th>
<th>Clinical Sensitivity</th>
<th>Analytical Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott</td>
<td>CIN2+</td>
<td>100%</td>
<td>96.0%</td>
</tr>
<tr>
<td>HC2</td>
<td>CIN2+</td>
<td>88.2%</td>
<td>90.0%</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Test</th>
<th>Disease</th>
<th>Clinical Sensitivity</th>
<th>Clinical Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott</td>
<td>CIN2+</td>
<td>90%</td>
<td>50%</td>
</tr>
<tr>
<td>HC2</td>
<td>CIN2+</td>
<td>95%</td>
<td>50%</td>
</tr>
<tr>
<td>Linear Array</td>
<td>CIN2+</td>
<td>92%</td>
<td>47%</td>
</tr>
</tbody>
</table>
## Additional Detection and Genotyping Assays

<table>
<thead>
<tr>
<th>Assay</th>
<th>Method</th>
<th>Primers</th>
<th>HPV types</th>
<th>IC</th>
</tr>
</thead>
</table>
| Linear Array (Roche)         | PCR and RLB                         | L1 gene PGMY09/11               | HR: 16, 18, 31, 33, 35, 39, 45, 51, 5253, 56, 58, 59, 66, 68, 70, 73, 82  
|                              |                                     |                                 | LR: 6, 11, 40, 42, 43, 44, 54, 55, 61, 62, 64, 67, 69, 70, 71, 72, 81, 83, 8482v, 89          | Low and high β-globin  |
| Inno-LiPA (Innogenetics)     | PCR and line probe assay             | SPF10                           | HR: 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, 82  
|                              |                                     |                                 | LR: 6, 11, 40, 43, 44, 54, 69, 71, 70, 74                                                   | HLA-DPB1               |
| Digene RH Test (Qiagen)      | PCR, reverse strip hybridization     | L1 gene GP5+/6+                 | HR: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82  
|                              |                                     |                                 |                                                                                               | yes                     |
| Digene LQ Test (Qiagen)      | PCR and bead based detect (LiquiChip)| L1 gene GP5+/6+                | HR: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82  
|                              |                                     |                                 |                                                                                               | yes                     |
| PapilloCheck (greiner bio-one)| PCR plus DNA chip hybridization     | E1 specific primers             | HR: 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, 82  
|                              |                                     |                                 | LR: 6, 11, 40, 42, 43, 44                                                                     | ADAT1                   |
## Additional Detection and Genotyping Assays

<table>
<thead>
<tr>
<th>Assay</th>
<th>Method</th>
<th>Primers</th>
<th>HPV types</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV4A ACE (Seegene)</td>
<td>Dual priming oligo PCR+ CE</td>
<td>Geno: 16,18 and Screening:</td>
<td>HR: 31,33,35,45,51,56, 58,59,66 67,70</td>
</tr>
<tr>
<td>CLART HPV 2 (Genomica)</td>
<td></td>
<td></td>
<td>LR: 6,11,42,43,44</td>
</tr>
<tr>
<td>GenoSquare (Kurbo)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GenoFlow HPV Array (DiagCor)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fHPV Typing (molGENTIX)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FTA cartridge</td>
<td></td>
<td>35 genotypes, low density array</td>
<td></td>
</tr>
<tr>
<td>Array based</td>
<td></td>
<td>23 genotypes, microarray</td>
<td></td>
</tr>
<tr>
<td>GenoArray</td>
<td></td>
<td>33 genotypes, amp and flow through hybridization</td>
<td></td>
</tr>
<tr>
<td>Polymer based chip</td>
<td></td>
<td>15 genotypes, PCR with fluorescent probes</td>
<td></td>
</tr>
<tr>
<td>Ultrasensitive genotyping assay</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

References:
- Lenselink CH. JCM 2009:47:2564
- Garcia-Sierra N. JCM 2009:47:2165
- Schenk T. JCM. 2009: 47:1428
- Schmitt M. JCM. 2010: 48:143
RNA Based Testing Methods
mRNA: Alternative to DNA Testing

- HPV DNA is present in transient infections, but very little E6/E7 mRNA is expressed
  - HPV DNA is detected by HPV DNA assays
  - Concentration of mRNA may be too low for APTIMA HPV to detect

- Too many “false positives” with regard to disease are identified with HPV DNA Tests
  - Episomal HPV DNA is present but infection regresses and no clinical disease is present
mRNA: Alternative to DNA Testing

- When HPV persists and integrates, over-expression of E6/E7 mRNA occurs
  - Infection is less likely to regress
  - Higher grade lesions and cancer may occur with HPV persistence
  - APTIMA HPV test detects over-expression of mRNA

- Detection of E6/E7 mRNA by APTIMA HPV may be more specific for detecting the progression of clinical disease
## E6/E7 mRNA Assays: Analytical Properties

<table>
<thead>
<tr>
<th>Test</th>
<th>Method</th>
<th>Targets</th>
<th>IC</th>
<th>LOD</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aptima HPV Assay (GenProbe) (Docter J. JCV:45:S39)</td>
<td>TMA</td>
<td>16,18,31,33,35,39,45,51,52,56,58,59,66,68</td>
<td>No</td>
<td>24-488 cps/rxn</td>
<td>6,11,42,43,44 53,61,71,81 99-100%</td>
</tr>
<tr>
<td>PreTect Proofer (Norchip) (Molden T, JVM 142:204)</td>
<td>NASBA</td>
<td>16,18,31,33,35</td>
<td>Yes</td>
<td>1-100 cells</td>
<td>16,18,31,33 45,6/11,35,51</td>
</tr>
<tr>
<td>NucliSENS EasyQ HPV v1 (bioMerieux) (Jeantet, D JCV:45:S29)</td>
<td>NASBA</td>
<td>16,18,31,33,35</td>
<td>Yes</td>
<td>230 to 3,000 cps/ml</td>
<td>6,52,58 limited</td>
</tr>
<tr>
<td>HPV OncoTect E6/E7 mRNA (incellDx)</td>
<td>Flow cytometry</td>
<td>Transforming cells E6/E7 mRNA in each cell</td>
<td>Yes</td>
<td>5-10 mRNA cps/cell</td>
<td></td>
</tr>
</tbody>
</table>
Norchip PreTect HPV Proofer

- Detection and typing of E6/E7 oncogene mRNA from class I carcinogens HPV 16, 18, 31, 33, 45
- U1A mRNA (cellular housekeeping gene) as intrinsic control to avoid false negatives due to sample degradation
- Each sample goes through 3 **duplex** NASBA amps
  - U1A + HPV 16
  - HPV 18 + 31
  - HPV 33 + 45
- 96 tests or 30 patient samples
- Cytobrush or Thin Prep collection
- Positive controls for all HPV types
- CE marked
NASBA and Molecular Beacons

- sense RNA oligo P1
- Reverse Transcriptase
- RNase H primer P2
- T7 RNA polymerase
- Fam Texas red
- reverse transcriptase
- RNase H oligo P1
- primer P2
- antisense RNA
- T7 RNA polymerase
Norchip PreTect Proofer Method

30 Samples

- HPV 16 + control at well 31
- HPV 18/31 + control at well 63
- HPV 33/45 + control at well 95
- Negative controls at wells 32/64/96

U1A+/HPV16- HPV18+/31+ HPV33-/45+

Fluorescence curves for each sample (example)
# Norchip PreTect Proofer HPV Test: Clinical Studies

## Molden T. 2005 Cancer Epid Biomarkers & Prevention:14:367

4,136 pts >30 yr with 2 yr follow up of HSIL

<table>
<thead>
<tr>
<th>Clinical Data:</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proofer</td>
<td>HSIL</td>
<td>52.0%</td>
<td>97.3%</td>
<td>10.3%</td>
</tr>
<tr>
<td>Gp5+/6+ PCR</td>
<td></td>
<td>64.0%</td>
<td>90.0%</td>
<td>3.7%</td>
</tr>
<tr>
<td>Proofer</td>
<td>CIN2+</td>
<td>85.7%</td>
<td>88.9%</td>
<td>92.3%</td>
</tr>
<tr>
<td>Gp5+/6+ PCR</td>
<td></td>
<td>92.9%</td>
<td>66.7%</td>
<td>81.3%</td>
</tr>
<tr>
<td>Abnor Cyto all</td>
<td></td>
<td>4.0%</td>
<td>Proofer HPV+ 3.0%</td>
<td>PCR HPV+ 4.4%</td>
</tr>
</tbody>
</table>

## Molden T. 2005 Int J Cancer 114:973

2 yr follow up of ASCUS or LSIL

<table>
<thead>
<tr>
<th>Clinical data:</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proofer</td>
<td>CIN2+</td>
<td>85.7%</td>
<td>84.9%</td>
<td>37.5%</td>
</tr>
<tr>
<td>Gp5+/6+ PCR</td>
<td></td>
<td>85.7%</td>
<td>50.0%</td>
<td>15.4%</td>
</tr>
<tr>
<td>Progression Risk</td>
<td>CIN2+</td>
<td>Proofer(+) vs (-) 69.8x</td>
<td>PCR(+) vs (-) 5.7x</td>
<td></td>
</tr>
</tbody>
</table>
# Norchip PreTect Proofer HPV Test: Clinical Studies

## Trope A. 2009 JCM:47: 2458: 643 pts >CIN2, 739 WNL

<table>
<thead>
<tr>
<th>HPV+</th>
<th>WNL</th>
<th>CIN2+</th>
<th>CIN2</th>
<th>CIN3/ACIS</th>
<th>ICC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proofer</td>
<td>3.3%</td>
<td>64.1%</td>
<td>50.4%</td>
<td>67.5%</td>
<td>76.9%</td>
</tr>
<tr>
<td>Amplicor</td>
<td>8.3%</td>
<td>96.4%</td>
<td>95.6%</td>
<td>97.0%</td>
<td>84.6%</td>
</tr>
</tbody>
</table>

## Varnai A. 2008 Oncology Reports 19:457: 66 women WNL to HSIL DNA+

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proofer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIN2+</td>
<td>81%</td>
<td>33%</td>
<td>85%</td>
<td>29%</td>
</tr>
<tr>
<td>CIN3+</td>
<td>95%</td>
<td>55%</td>
<td>81%</td>
<td>86%</td>
</tr>
<tr>
<td>Progression</td>
<td>78.7%</td>
<td>60%</td>
<td>37%</td>
<td>90%</td>
</tr>
</tbody>
</table>

## Keegan H. 2009 JVM 155:61: 299 LBC WNL-CIN3

<table>
<thead>
<tr>
<th>HPV+</th>
<th>WNL</th>
<th>BNA</th>
<th>CIN1</th>
<th>CIN2</th>
<th>CIN3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proofer</td>
<td>3.3%</td>
<td>14.7%</td>
<td>37%</td>
<td>67%</td>
<td>83%</td>
</tr>
<tr>
<td>HC2</td>
<td>8.3%</td>
<td>47.1%</td>
<td>83%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Sens/spec for CIN2+
- Proofer: 71.4%/75.8%
- HC2: 100%/43.7%
bioMerieux NucliSENS EasyQ HPV

- NASBA and molecular beacon detection
- Modified version of Norchip Assay
- Detects E6/E7 mRNA of HPV16, HPV18, HPV31, HPV33, HPV45
- NucliSENS easyMAG extraction
- Internal control U1A
- CE marked
bioMerieux NucliSENS EasyQ E6/E7: Clinical Studies

CIN2+ HSIL

- Sensitivity
- NPV
- Specificity
- PPV

CIN2+

- Sensitivity
- NPV
- Specificity
- PPV

Halfon P. 2010 JCV:47:177
140 pts referred for colposcopy

Cattani P. 2009 JCM:47:3895
180 pts referred for colposcopy
Gen-Probe Aptima® HPV Assay Overview

- CE marked for diagnostic screening of liquid based cytology (LBC) samples
- Not FDA approved for sale in the U.S.
- Multiplex assay: Single tube format
- Targets HPV E6/E7 mRNA transcripts
  - Detects 14 High Risk (HR) types
    - 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68
  - No cross-reaction with 5 Low Risk (LR) types
    - 6, 11, 42, 43, 44
- Typing assay for 16, 18 and 45
- Run on fully automated TIGRIS
Aptima HPV: Three Technologies

1. Target capture specimen processing
2. Transcription-Mediated Amplification (TMA)
3. Dual Kinetic Assay (DKA)
Dockter J. 2009 JCV:45:S55
800 pts referred for colposcopy

*p = <0.0001
Shenzhen Cervical Cancer Screening Program I

<table>
<thead>
<tr>
<th>SCREENING TEST</th>
<th>SENSITIVITY FOR &gt;=CIN 2 (% CI)</th>
<th>SPECIFICITY FOR &gt;=CIN 2 (% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SurePath&gt;ASCUS</td>
<td>66.7 (46.0, 83.5)</td>
<td>94.3 (93.2, 95.3)</td>
</tr>
<tr>
<td>HC-II</td>
<td>88.9 (70.8, 97.6)</td>
<td>81.8 (80.0, 83.4)</td>
</tr>
<tr>
<td>AHPV</td>
<td>100 (87.2, 100)</td>
<td>89.2 (87.7, 90.5)</td>
</tr>
</tbody>
</table>

Using McNemar’s Chi-square the sensitivity of AHPV differs from that of SurePath P=0.004 and the specificities of these tests differ from each other P<0.0001

Wulan N., et al. ShenCast I, Eurogin 2010
Referral Population - Sensitivity Comparison: Canadian vs UK/French/German Studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample Size</th>
<th>Prevalence of CIN 2+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratnam et al.</td>
<td>n = 1418</td>
<td>Prevalence of CIN 2+ = 401 (28.3%)</td>
</tr>
<tr>
<td>Szarewski et al.</td>
<td>n = 953</td>
<td>Prevalence of CIN 2+ = 273 (28.6%)</td>
</tr>
<tr>
<td>Dockter et al.</td>
<td>n = 753</td>
<td>Prevalence of CIN 2+ = 141 (18.7%)</td>
</tr>
<tr>
<td>Clad et al.</td>
<td>n = 424</td>
<td>Prevalence of CIN 2+ = 252 (59.4%)</td>
</tr>
<tr>
<td>Cuschieri et al.</td>
<td>n = 1470</td>
<td>Prevalence of CIN 2+ = 343 (20.3%)</td>
</tr>
</tbody>
</table>

Courtesy of S. Ratnam
IPV Montreal 2010
Referral Population - Specificity Comparison: Canadian vs UK/French/German Studies

<table>
<thead>
<tr>
<th>Study</th>
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<tr>
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<td>424</td>
<td>252 (59.4%)</td>
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<tr>
<td>Cuschieri et al.</td>
<td>1470</td>
<td>343 (20.3%)</td>
</tr>
</tbody>
</table>

Courtesy of S. Ratnam
IPV Montreal 2010
DNA vs RNA Detection of CIN2+

Hybrid Capture II

Amplicor

Linear Array

APTIMA

Genomica

Sensitivity

Specificity

Szarewski, A., et. al. Cancer Epidemiol Biomarkers
Prev 2008;17(11) 2008
DNA vs RNA Detection of CIN3+

Sensitivity vs Specificity graph:
- Hybrid Capture II
- Amplicor
- APTIMA Linear Array
- HPV-Proofer
- Genomica
- p16^{INK4a}

References:
Future Challenges
Special Commentary: Patient Safety and the Next Generation of HPV DNA Tests


- Clinical sensitivity and clinical specificity are important to patient safety
- Must be considered in the context of using current and future assays
- Exquisite analytical sensitivity does not increase the clinical sensitivity of an HPV test
- Does result in excessive test positivity and decreased clinical specificity
- Potential to result in unnecessary: follow-up, diagnostic procedures, and treatment of healthy women
Alternative Sample Types

- Invasive anal squamous cell carcinoma: Annual screening in HIV+ men by cytology and HPV?

  - 91% HIV+ and 57% HIV- MSM had anal HPV
  - HIV+ men sensitivity of abnormal cytology to detect high-grade anal neoplasia was 87% and in HIV- MSM: 55%
  - HIV- men, 9/20 cases of high-grade anal neoplasia would have been missed because cytology was negative
  - Addition of HPV+ increased sensitivity for the combination to 90%

  - Presence of AIN 2/3 in HIV+ men was associated with multiple HPV genotypes, HPV genotypes 16 and 31, and HPV 16 viral load
Conclusions

- HPV testing is essential for the detection of clinically relevant cervical disease (CIN3+)
- A wide range of HPV detection and genotyping assays are available
- Assays must be thoroughly validated to demonstrate actual clinical performance
- Clinicians and laboratorians must understand the clinical performance of the assays and critically evaluate and compare clinical trial data
- Assay variations and characteristics may change future testing algorithms
Thank you