Recent Advances in the Use of HPV mRNA Assays for Cervical Disease Detection

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Presentation Overview

• Cervical Cancer Overview
• HPV Overview and Natural History
• Cervical Cancer Screening
• Molecular HPV Testing
• HPV mRNA Testing
  – APTIMA HPV Assay
ESTIMATED NUMBER OF CERVICAL CANCER CASES
in 2002: 490,000

N. AMERICA 14,670
C.-S. AMERICA 71,862
EUROPE 59,931
AFRICA 78,897
ASIA 265,884

Age-adjusted incidence rates per 100,000 women per year
Cervical Cancer Facts Worldwide

• 99.7% of cervical CA due to HPV infection
• 2.30 billion women worldwide over 15 years of age
  – 300 million harbor genital HPV infection at any point in time
• 1.4 million women
  – living with clinical cervical pre-cancer/cancer
  – lower cervical cancer rates in U.S. and EU due to cervical screening programs
Human Papillomavirus
Papillomaviruses

- Family of DNA viruses
- Highly diverse and occur in almost all mammals and birds
- Over 100 Human Papillomavirus (HPV) types have been identified
  - At least 30 types are sexually transmitted

Wright, T.C., et. al. "Clinical Uses of HPV DNA Testing" ASCCP 2006
Human Papillomavirus Virus (HPV)

- At least 14 of the HPV genotypes are classified as “high-risk”
- Persistent infection of these 14 types is the causative agent of cervical cancer.
- HPV-16 & HPV-18 are the most prevalent carcinogenic types
  - Account for approx. 70-80% of cervical cancer worldwide
HPV Types in Cervical Cancer by Region

15 types are associated with cervical cancer
HPV Infection

- 30-60% of sexually active men & women are infected with genital HPV at some point in their life.
- Infections are largely asymptomatic.
- In transient HPV infections, the virus may be cleared or reduced to undetectable levels.

Burden of HPV-Related Diseases in Women

300 million
HPV infection – no abnormality

30 million
Low grade lesions

30 million
Genital warts

10 million
High grade cervical lesions

500 K
Cervical cancer
HPV Genome

• Circular DNA Genome ~8000 nucleotides divided into 2 regions:
  • Early region – viral replication
  • Late region = viral capsid

• E1, E2, E4, and E5 required for viral replication

• E6 and E7 encode viral oncoproteins

• L1, L2 encode capsid proteins

• URR (Upstream Regulatory Region) contains the genetic sequences which control transcription of the viral genome.
Viral Integration

Initial HPV infection

- Low levels of E6/E7 mRNA expression

HPV DNA integration

- Increased E6/E7 mRNA expression
- Increased probability of progression to disease
Cervical Cancer Screening
Types of Cervical Cancer

• Squamous Cell Carcinoma
  – 85% of cervical cancers fall in this category
  – Arises from squamous epithelium that covers the visible part of the cervix.
  – Well established progression through premalignant changes before a cancer develops

• Adenocarcinoma
  – Arises from the glandular lining of the endocervical canal.

• Takes ~5-15 years for the initial HPV infection to result in cervical cancer
Persistent Infection

• Defined as “HPV positive test at 2 different time points”
• Infections frequently lead to micro-lesions or proliferations not visible to the eye
  – e.g. LSIL (Low-grade squamous intraepithelial lesions)
• Cause cellular changes that lead to abnormal tissue growth (genital warts/cervical lesions)
  – greatest risk to develop high-grade CIN & invasive cancer
  – more common with high-risk than low-risk types
Latent Infection

- Infections that do not produce lesions are referred to as *latent infections*
- Tend to coexist with host for long periods of time
- Infections are largely asymptomatic
- Infections are largely transient
- Virus may be cleared or reduced to undetectable levels

Wright, T.C., et. al. “Clinical Uses of HPV DNA Testing” ASCCP 2006
Conventional Methods for Cervical Cancer Screening

<table>
<thead>
<tr>
<th>Pap Smear (Cytology)</th>
<th>Colposcopy</th>
<th>Tissue Biopsy (Histology)</th>
</tr>
</thead>
</table>
| ASC-US               | Acidic wash of cervix to visualize lesions with colposcopy instrument | CIN 1  
Atypical Squamous Cell-Undetermined Significance  
Cervical Intraepithelial Neoplasia (Mild Dysplasia) |
| LSIL                 |            | CIN 2 & CIN 3  
Low-grade squamous intraepithelial lesion  
(Moderate to Severe Dysplasia) |
| HSIL                 |            |                           |

CIN 2 & CIN 3 (Moderate to Severe Dysplasia)

CIN 1  
Cervical Intraepithelial Neoplasia (Mild Dysplasia)

ASC-US  
Atypical Squamous Cell-Undetermined Significance

LSIL  
Low-grade squamous intraepithelial lesion

HSIL  
High-grade squamous intraepithelial lesion
Pap Testing

- Pap testing alone has significantly reduced mortality due to cervical cancer (70% since 1941)
- Limitations of a single Pap test
  - Suboptimal sensitivity (30% to 70%)
  - Limited reproducibility
  - Subjective diagnosis
  - Equivocal Pap test results → ASC-US (atypical squamous cells of undetermined significance)
Liquid-based Pap Testing

• Began in 1996 with the ThinPrep Pap Test by Cytyc.
• Liquid based Pap (LBC) tests have higher throughput processing than pap smears.
• Infectious organisms such as CT, GC, Trich & HPV can also be tested out of the ThinPrep vial.
• SurePath LBC media is a second type of media which is also widely used.
Dysplastic changes

- **Normal Epithelium**: 90%
- **HPV Infection**: 30 - 40%
- **CIN1**: 10 - 20%
- **CIN2**: 7 - 10 years, 30-80%
- **CIN3**: 2 - 4 years, 30 - 40%
- **Carcinoma**: 0 - 24 months, 30%
Cervical Cancer Progression Model: Non-infected

Normal cervical epithelium
Cervical Cancer Progression Model: HPV Infection

- High levels of HPV DNA
- Low levels of E6/E7 mRNA
- HPV infected cervical cells
Cervical Cancer Progression Model: Progression to CIN1

- High levels of HPV DNA
- Low levels of E6/E7 mRNA
- CIN 1 or LSIL (low grade intraepithelial lesions)
Cervical Cancer Progression Model: Pre-cancer CIN2

- High levels of HPV DNA
- Higher levels of E6/E7 mRNA
- CIN2 or HSIL (high-grade intraepithelial lesions)
Cervical Cancer Progression Model: Pre-cancer CIN3+

High levels of HPV DNA

Higher levels of E6/E7 mRNA

CIN3+ or HSIL (high-grade intraepithelial lesions)
Cervical Cancer Progression Model: Invasive Cancer

High levels of HPV DNA

Highest levels of E6/E7 mRNA

Cervical carcinoma
Molecular HPV Testing
Molecular HPV Testing

Screening Tests:
- Digene HPV (Qiagen)
- APTIMA HPV
- Roche Cobas
- NorChip (Bmx)
- Cervista (Hologic)
- Abbott

Genotyping Tests:
- Roche Linear Array
- Genomica
- Greiner Bio-one
- Innogenetics
Molecular HPV Testing Uses

• HPV Testing with Abnormal Cytology (ASC-US Triage or HPV “reflex”)
  – Determine the need for colposcopy
• HPV Testing as an adjunct to Cytology
  – HPV test ordered at the same time as Pap
• HPV Testing as the primary screen
  – HPV test done first– reflex to cytology if positive
• Post treatment follow up
Age Consideration for HPV Screening

• Adjunct HPV Testing is only recommended in women over 30 (in U.S.).*
  – If under 30, HPV infection is high, but cancer prevalence is low
    • 25% of women in their early 20’s are high-risk HPV positive but less than 0.002% of them have cervical cancer

*American Cancer Society
Digene HPV Test

- First FDA–approved HPV test for:
  - Adjunct screening, in conjunction with a Pap, of women age 30 years and older; and
  - Triage of women of any age with ASC–US Pap results.

- US National guidelines recommend that high-risk HPV testing be used in conjunction with the Pap for improved detection of cervical disease and cancer.
Performance Summary of HPV DNA Testing vs. Cytology

- HPV testing is more sensitive than cytology
- Cytology is more specific than HPV testing
- Both tests together provide the best sensitivity
- Primary HPV screening with reflex to cytology may occur in the future:
  - Screen with the most sensitive test, reflex to most specific test
  - Cost savings may occur as screening intervals could be increased
HPV mRNA Testing
HPV mRNA Testing Rationale

• Transient infections: HPV DNA is present but very little E6/E7 mRNA is expressed
  – HPV DNA is detected by HPV DNA assays
  – Concentration of mRNA may be too low for HPV mRNA tests 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
HPV mRNA Testing Rationale

• Persistent infections: HPV integrates, over-expression of E6/E7 mRNA occurs.
  – Infection is less likely to regress
  – Higher grade lesions and cancer may occur with HPV persistence

• Detection of E6/E7 mRNA may be more specific for assessing progression of clinical disease
First Commercial E6/E7 RNA Assay

HPV Proofer (Norchip; now also BioMerieux EasyQ HPV)
  - NASBA amplification prior to detection of **HPV 16,18,31,33,45** via molecular beacons
  - Resolves type, qualitative, internal control: (U1A mRNA)

Primer 1 + T7
promoter recognises
ss RNA

AMV, RnaseH,
AMV, primer 2

Double stranded DNA
- functional promoter

T7 RNA pol

Active production
of transcripts

Can be used for routinely collected cervical cytology samples

Fluorescent detection of transcripts by molecular beacons
## Studies using NASBA Technology for E6 and E7 Detection

<table>
<thead>
<tr>
<th>Author</th>
<th>Population studied</th>
<th>Specimen type</th>
<th>Assay</th>
<th>Pos (%)</th>
<th>Pos (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kraus 2006</td>
<td>Cancers</td>
<td>Formalin Fixed Biopsy</td>
<td>PreTect HPV Proofer</td>
<td></td>
<td>199/204</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(98%)</td>
</tr>
<tr>
<td>Lie 2005</td>
<td>Referral population</td>
<td>Cervical liquid based cytology</td>
<td>PreTect HPV Proofer + further 3 types</td>
<td>225/291</td>
<td>20/20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(77%)</td>
<td>(100%)</td>
</tr>
<tr>
<td>Molden 2005</td>
<td>Screening Population</td>
<td>Cervical scrape in buffer</td>
<td>PreTect HPV Proofer</td>
<td>13/14</td>
<td>(93%)</td>
</tr>
<tr>
<td>Szarewski 2008</td>
<td>Referral population</td>
<td>LBC</td>
<td>PreTect HPV Proofer</td>
<td>196/266</td>
<td>(73.6 %)</td>
</tr>
<tr>
<td>Keegan 2008</td>
<td>Convenience sample of all cytologic grades</td>
<td>LBC</td>
<td>PreTect HPV Proofer</td>
<td>60/84</td>
<td>(71.4%)</td>
</tr>
<tr>
<td>Trope 2009</td>
<td>Gynaecology clinics, high grade samples</td>
<td>LBC</td>
<td>PreTect HPV Proofer</td>
<td>412/643</td>
<td>(64.1%)</td>
</tr>
<tr>
<td>Cattani 2009</td>
<td>HC2+ convenience sample of all cytologic grades</td>
<td>LBC</td>
<td>EasyQ HPV test</td>
<td>57/66</td>
<td>(86.3%)</td>
</tr>
<tr>
<td>Halfon 2009</td>
<td>Colposcopy clinic</td>
<td>LBC</td>
<td>EasyQ HPV test</td>
<td>28/37</td>
<td>(76%)</td>
</tr>
</tbody>
</table>
APTIMA® HPV Assay

• Nucleic acid amplification assay utilizing APTIMA technology
  – Target capture specimen processing
  – Transcription-Mediated Amplification (TMA)
  – Dual Kinetic Assay (DKA) detection technology

• Qualitatively detects HPV E6/E7 mRNA of 14 high risk HPV subtypes

• CE marked in Europe for diagnostic screening of liquid based cytology (LBC) and APTIMA cervical sampler specimens
APTIMA® HPV Assay Overview

• Multiplex assay – qualitatively detects 14 HPV types in a single tube
  – 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

• The assay will only detect presence or absence of HR types
  – If positive, the assay will not specify which types are present
Target Capture

Poly-T oligomer bound to magnetic particle

Capture oligomer

Target sequence
Why Target Capture?

**Target Capture technology is designed to:**

- Virtually eliminate false negatives by removing inhibitors
- Simplify sample processing
- Allow the use of large sample volumes
- Accommodate numerous specimen types
- Allows testing of multiple specimen types in same run
- Reduce contamination by targeting a different region of the mRNA than the amplicon produced by TMA
Detection by Dual Kinetic Assay (DKA) Technology

- Modification of Hybridization Protection Assay (HPA) Technology
- Two unique acridinium ester labels with different light-off kinetics on different DNA probes
  - Allows simultaneous detection of two different nucleic acid targets
APTIMA® HPV Assay

- The APTIMA HPV assay:
  - Follows the procedure for the APTIMA Family of products
    - Difference: Addition of calibrators and internal control
  - Has a time to first result of 3.5 hours
  - Has high throughput on the TIGRIS platform
TIGRIS® DTS® System Design Features

- Fully automated: Sample processing, amplification, detection and results reporting
- Single tube assay process, 180 bar coded primary specimen tubes on board
- Penetrable tube caps utilized
- Automated bar code scanning to create work list
- Continuous batch loading of specimens with reagents/fluids on board for 1,000 tests
- System throughput:
  - 500 tests/8.5 hours; 1,000 tests/13.5 hours
APTIMA® HPV

• Specimen
  – Compatible with liquid-based cytology (LBC) cervical specimens
    • CE marked for Cytyc ThinPrep LBC specimens
  – 1 mL of Cytyc LBC
    • transferred to 2.9 mL STM
    • 30 day RT storage stability

• Internal control
  • controls for inhibition and technician error
APTIMA® HPV Studies: Assay Performance
Comparison of Predictors for High-Grade Cervical Intraepithelial Neoplasia in Women with Abnormal Smears

A Szarewski, et. al.
Cancer Epidemiol Biomarkers Prev. 2008;17(11).
November 2008
Aim

To evaluate and compare the sensitivity and specificity of several adjunctive tests for the detection of high-grade disease (CIN2+ and CIN3+).
Methods

• Women referred for colposcopy: high disease rate
• 953 women aged 18 to 72
• At referral:
  – LBC sample taken for cytology and other tests

Tests evaluated:

(From a liquid PreservCyt® sample)

<table>
<thead>
<tr>
<th>Test</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repeat cytology</td>
<td></td>
</tr>
<tr>
<td>Hybrid Capture II</td>
<td>(Digene)</td>
</tr>
<tr>
<td>Amplicor</td>
<td>(Roche)</td>
</tr>
<tr>
<td>HPV-Proofer</td>
<td>(Norchip)</td>
</tr>
<tr>
<td>APTIMA HPV assay</td>
<td>(Gen-Probe)</td>
</tr>
<tr>
<td>Linear Array</td>
<td>(Roche)</td>
</tr>
<tr>
<td>Clinical Arrays</td>
<td>(Genomica)</td>
</tr>
<tr>
<td>p16\textsuperscript{INK4a} immunocytochemistry</td>
<td>(on a subset)</td>
</tr>
</tbody>
</table>

Hybrid Capture II

Detection of CIN3

Sensitivity vs. Specificity

Ideal Test

Hybrid Capture II

Amplicor

Linear Array

APTIMA

Genomica

p16\textsuperscript{INK4a}

HPV-Proofer

Detection of CIN3

Predictors Study Conclusions

• Five tests have sensitivity ≥ 95%
  – Amplicor
  – APTIMA®
  – HC II
  – Linear Array
  – repeat cytology

• Of these 5 tests, APTIMA shows best specificity

Summary of HPV Referral Studies

AHPV is sensitive for detecting disease (CIN2+)

Ratnum Cuscheri Clad Dockter Smith

AHPV Sensitivity

HC2 Sensitivity
Summary of HPV Referral Studies

AHPV is more specific for detecting disease (CIN2+)
EVALUATION OF ONCOGENIC HUMAN PAPILLOMAVIRUS RNA AND DNA TESTS WITH LIQUID BASED CYTOLOGY IN PRIMARY CERVICAL CANCER SCREENING (THE FASE STUDY)

J. Monsonego, et. al.

In-Press: Int’l J. Cancer
Aims: French APTIMA Screening Evaluation (FASE) Study

- Assess the performance of the APTIMA® HPV Assay for detection of high-risk mRNA in comparison with the Hybrid Capture 2 Assay for detection of high-risk HPV DNA.

- Compare the HPV assays either as stand-alone tests (primary screening tools) or in combination with LBC (adjunct screening tools), for the detection of high-grade CIN lesions in a screening population of 5000 women in France.
Materials and Methods

FASE Study

• Regional, cross-sectional, cervical cancer screening study of 5006 women (age 20-65 years) for detection of cervical intraepithelial neoplasia (CIN)

• Comparison of the APTIMA HPV mRNA assay with the Hybrid Capture 2 HPV DNA assay using ThinPrep liquid based cytology specimens (LBC)

• HPV assays evaluated either as stand-alone tests (primary screening, or in combination with LBC (adjunct screening)

• Women cytologically abnormal or positive for either HPV test are sent for colposcopic evaluation and biopsy
FASE Opportunistic Screening Algorithm

Opportunistic screening study
17 centers
N=4,481 women

N=52 women excluded
32 missing RNA test
50 missing DNA test

Valid HPV DNA test, HPV RNA test and cytology:
N=4,429

Normal cytology and HPV DNA and HPV RNA
N=3511

End of Study: N=3020
14% Random sample referred to colposcopy N=491

End of Study
No histology obtained: N=213

Histology normal (<CIN-1+): N=160

Histology abnormal: (CIN-1+): N=118

LEEP or punch biopsy histology: N=278

Histology normal: (<CIN-1+): N=336
Histology abnormal: (CIN-1+): N=499

End of study
No colposcopy conducted: N=78

Colposcopy conducted N=840

End of study
No histology obtained: N=5

Colposcopy conducted N=840
Estimated prevalence of HPV infection by HPV DNA and HPV RNA stratified by histology

N=1113 biopsies
### Screening Test Performance (CIN3+)

\( N(\text{CIN3+}) = 22 \)

<table>
<thead>
<tr>
<th>Test</th>
<th>%Sensitivity (95%CI)</th>
<th>%Specificity (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LBC ASCUS</td>
<td>73.3 (55.6-91.0)</td>
<td>90.8 (91.2-93.0)</td>
</tr>
<tr>
<td>APTIMA HPV</td>
<td>95.7 (85.0-100)</td>
<td>90.3 (89.4-91.2)</td>
</tr>
<tr>
<td>Hybrid Capture2</td>
<td>95.3 (83.9-100)</td>
<td>84.9 (83.8-86.0)</td>
</tr>
</tbody>
</table>
HPV Positivity by Histology Result
Among subjects referred to colposcopy N=1331

HC2 = 65% more false positives
Conclusions
FASE Study Analysis

- Both the AHPV and HC2 assays are more sensitive than cytology
- The AHPV assay is as specific as LBC
- Fewer specimens tested positive with the AHPV assay compared with the HC2 assay, especially in women with ≤CIN1
- The AHPV assay has statistically the same sensitivity, but higher specificity and PPV than the HC2 assay for detection of CIN2+
- The AHPV E6/E7 mRNA assay may be more useful than HPV DNA assays for detecting cervical disease
Performance Summary of Current HPV Tests

• HPV DNA tests have high sensitivity but low specificity:
  – Detect episomal virus that has not integrated
  – Integration is necessary for cervical disease
  – Many transient infections are detected that will not cause cervical disease

• APTIMA HPV has equivalent clinical sensitivity but higher specificity compared to HPV DNA tests:
  – Detects mostly integrated, active virus leading to cervical disease
  – Detects all 14 subtypes
Conclusions

The APTIMA® HPV assay:

• Utilizes proven second generation nucleic acid target amplification technology
• Has instrumentation and workflow advantages that makes the assay highly automated and easy to run
• Detects HPV E6/E7 mRNA with equivalent clinical sensitivity and higher specificity than current HPV DNA tests