HPV E6/E7 mRNA Test

The OncoTect® HPV Test

A one-sure test for Cervical Screening & Triage

India awaits QII, 2013

Dinesh Gupta, PhD
Evolution of Ca Cx Screening

CERVICAL SCREENING MKT

1998
HPV HC2
Strong footprint in cancer screening

2007-10
LBC & other HPV Tests
Marginal clinical benefits

2010
HPV Genotype

2013
HPV E6E7 mRNA OncoTect®
Big leap, filling deficiencies (^
Specificity)

Other HPV, LBC limited impact
WHY DOES a Ca Cx Detection require a new test?

- “Low specificity of the current HPV DNA Testing largely attempted justified by high prevalence of HPV among younger women, but may be due to cross reactivity with non-oncogenic or the HPV types not included in the high risk panels!”

- “although cytological screening is effective in preventing the more common squamous-cell carcinoma of the cervix, it is insufficient for adenocarcinoma” Sasieni P. The Lancet, 2001; 357:1490-3

- ... so has HPV DNA Testing alone!

- “incidence of adenocarcinoma, which used to account for 10-15% of all cervical cancers, has been steadily increasing in young women, even as the overall incidence of cervical cancer has declined” Liu et al. CMAJ, 2001; 164(8):1151-2

- ... in some population settings however, or attributed to types 18, 45. Vinh-Hung V, et al. BMC Cancer 2007; 7:164-176

- Ad Ca is more aggressive and invasive than SCC, yet it is under-diagnosed...
CROSS REACTIVITY with some HPV types

According to hc2 PI:

“… small amount of cross-hybridization between HPV types 6 and 42 (low risk types) and the high risk probe group.”

This is seen as a low positive HR hc2 result that is negative for PCR yet positive on hc2 for Low Risk types (if tested).

“… hc2 High Risk HPV Probe has been shown to cross-react with HPV types 40, 53 and 66 … and there is insufficient evidence to establish the exact correlations between infection with these types and development of high grade disease.”
CROSS REACTIVITY: issues dealt with so far

“Cross-reactivity unintended but fortunately relate to greater sensitivity of hc2 with types that occasionally might cause cancer”. (Schiffman et al, Am J Clin Path 2005)

“cross-reactivity in fact has improved the screening performance by hc2 among cytology normal women as the result of increased sensitivity of histological ≥CIN 2” (Castle et al, Cancer Epidemiology, Biomarkers and Prevention 2002)

The clinical sensitivity of hc2 is validated and well published with results showing clinical sensitivity for identifying women at risk of CIN2/3+ endpoints from 97-100%. (Hasselink et al, Cancer Cytopathology 2004, Cuzick et al, The Lancet 2003)
ABOUT The Test (OncoTect™) - “game changer”

- provides **QUANTITATIVE** information on two levels:
  - the **quantity** of E6/E7 overexpression inside each cell and
  - the **percentage** of cells that overexpress E6/E7 mRNA.

- **MORE PREDICTIVE** for identifying precursors of cervical cancer. Most HR HPV infections will not lead to cancer as the viral DNA detected is in the **episomal (non-integrated)** form.

- Over-expression of E6E7 gene mRNA co-relates well with clinical **CIN detection**; offers **ADDITIONAL ADVANTAGE** of cell-cycle analysis and morphometric immunophenotyping analysis.

- Can use most **LBC media**, viz. TP, SP, LP. RNA detectable ~12 months of collection. **AT PAR SENSITIVITY, SUPERIOR SPECIFICITY**.

- **DIFFERENTIATES** between SCC & Ad Ca

- Ideal assay for ca of other sites, e.g. HNSCC

The HPV OncoTect™ E6, E7 mRNA Kit is a unique detection method that measures both the number of transforming cells and the quantity of E6, E7 mRNA in each cell. These two measurements precisely assess the overexpression of E6, E7 mRNA in routine patient samples collected in ThinPrep® and Surepath™ vials to further refine accuracy and specificity of HPV testing.

Technology simplified:
- No requirement for extraction of nucleic acid, eliminating cross contamination
- Results available in 3 hours for fast turnaround time
- Variable batch sizes (24 or 96 specimens)
- Result output is clearly stated to indicate positive or negative
- Adaptable on most Flowmeters

Clinical Performance of HPV OncoTect™ E6, E7 mRNA Kit:
- Equivalent clinical sensitivity to HR HPV DNA Tests (95% for ≥ CIN2)
- Significant increase in specificity
- Unique specimen adequacy feature:
  > QUANTIFIES number of ectocervical and endocervical cells
  > Quantifies the presence of obscuring inflammatory cells
How HPV Causes Carcinoma

- **Episomal HPV DNA**
- **Integrated HPV DNA**
- **Linear form**

- **Viral Integration**
How HPV Causes Carcinoma

- Viral Integration
- E6 E7 mRNA Upregulation
How HPV Causes Carcinoma

- Viral Integration
- E6 E7 mRNA Upregulation
- Oncoprotein Production

E6 E7 Oncoproteins

DNA
How HPV Causes Carcinoma

- Viral Integration
- E6 E7 mRNA Upregulation
- Oncoprotein Production
- Inactivation Of Cellular Tumor Suppressors

DNA

RB

p53
E6 E7 mRNA is a Genotype Independent Biomarker

Ubiquitous Presence of E6 and E7 Transcripts in Human Papillomavirus-Positive Cervical Carcinomas Regardless of Its Type

Shunsuke Nakagawa,* Hiroyuki Yoshikawa, Toshiharu Yasugi, Mami Kimura, Kei Kawana, Koji Matsumoto, Manabu Yamada, Takashi Onda, and Yuji Taketani

Department of Obstetrics and Gynecology, University of Tokyo, Faculty of Medicine, Bunkyo-ku, Tokyo, Japan

And Quantifying E6 E7 mRNA is the Key

Bright Field

Fluorescence

CIN 1

CIN 3

*Durst et al. Virology, 1992
HPV OncoTect® Performance Attributes

**Performance**
- Unmatched high specificity compared to biopsy
  - Cell type differentiation between squamous cell and adenocarcinoma
  - Effective in women < 30, unlike FDA-approved tests
  - Highest performance for AIN of any assay

**Workflow simplicity and speed**
- Benchtop instruments
- Fast process time: < 4 hours

**Economics**
- Price to lab
- No repeat testing or reflex HPV testing

**Clinical gold standard**

CIN2 +

<table>
<thead>
<tr>
<th>Disease</th>
<th>Sensitivity</th>
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<tbody>
<tr>
<td>CIN2</td>
<td>86%</td>
</tr>
<tr>
<td>CIN3</td>
<td>94%</td>
</tr>
<tr>
<td>Cancer</td>
<td>100%</td>
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</tbody>
</table>

12,000 patient clinical study biopsy correlation

*HPV OncoTect® identifies disease, not risk of disease*
# OncoTect™: hc2 – head to head

<table>
<thead>
<tr>
<th>hc2 Test</th>
<th>OncoTect test</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Qualitative or Semi-quant.</td>
<td>• QUANTITATIVE- cell-to-cell overexpression as well as overall tissue overexpression of E6/E7 oncogenes.</td>
</tr>
<tr>
<td>• A cocktail test for 13 HR types</td>
<td>• ALL TYPES OF HPV INFECTED CELLS that overexpress E6/E7 oncogenes</td>
</tr>
<tr>
<td>• Biological specimen with Sample Pre-treatment (LBC medium with sample conversion buffer)</td>
<td>• Biological specimens without Sample Prep step (Any LBC medium without pre-prep step).</td>
</tr>
<tr>
<td>• Sensitivity: 90%+ most studies.</td>
<td>• Sensitivity: 90%+ most studies</td>
</tr>
<tr>
<td>• Specificity: 25-28%.</td>
<td>• Specificity: 92%+</td>
</tr>
<tr>
<td>• Time to test- 4.5 hrs</td>
<td>• Time to test- 3.5 hrs</td>
</tr>
<tr>
<td>• Batch test</td>
<td>• Single-to-batch test</td>
</tr>
<tr>
<td>• Limited Features software</td>
<td>• Open feature software allows to study grouping, gating, morphometric changes.</td>
</tr>
<tr>
<td>• No specimen adequacy feature</td>
<td>• Unique specimen adequacy feature</td>
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### OncoTect™: other E6/E7 mRNA Tests – head to head

<table>
<thead>
<tr>
<th>Other HPV mRNA Tests</th>
<th>OncoTect test</th>
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<tbody>
<tr>
<td>• PCR-based. Requires viral DNA isolation and amplification.</td>
<td>• Flowmetry based. Biological specimen collected for LBC without pre-treatment.</td>
</tr>
<tr>
<td>• Skill &amp; reagent quality dependant</td>
<td>• User-friendly</td>
</tr>
<tr>
<td>• Qualitative</td>
<td>• Quantitative</td>
</tr>
<tr>
<td>• 14 to 15 HPV HR Types</td>
<td>• Any type of cell infected with HPV HR types.</td>
</tr>
<tr>
<td>• No disease threshold</td>
<td>• Above 2% cells that over-express E6/E7.</td>
</tr>
<tr>
<td>• Analytical Sensitivity varies for HR HPV types</td>
<td>• Analytical Sensitivity does not vary for any oncogenic HPV types</td>
</tr>
<tr>
<td>• Specificity: 40-42%</td>
<td>• Specificity: 92%+</td>
</tr>
<tr>
<td>• Tedious, requires PCR Lab</td>
<td>• Convenient, open lab.</td>
</tr>
<tr>
<td>• Manufacturer bound closed system</td>
<td>• Open system Flow Cytometer</td>
</tr>
<tr>
<td>• Relatively expensive and less reliable for reproducibility.</td>
<td>• Less expensive and reproducible intra-sample, inter-lab, different time samples etc</td>
</tr>
<tr>
<td>• Many choices</td>
<td>• One of the proprietary technology.</td>
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</table>
# Understanding HPV Genome - ORFs

<table>
<thead>
<tr>
<th>HPV Gene</th>
<th>Broad Functionality</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>URR</strong></td>
<td>Regulatory control of transcription, replication, and host interactions</td>
</tr>
<tr>
<td>L1</td>
<td>Major capsid protein</td>
</tr>
<tr>
<td>L2</td>
<td>Minor capsid protein</td>
</tr>
<tr>
<td>E1</td>
<td>Viral replication and maintenance of viral episome</td>
</tr>
<tr>
<td>E2</td>
<td>Transcriptional regulation and cofactor for replication</td>
</tr>
<tr>
<td>E4</td>
<td>Keratin interactions and viral shedding</td>
</tr>
<tr>
<td>E5</td>
<td>Growth factor receptor interactions and signal transduction</td>
</tr>
<tr>
<td><strong>E6 &amp; E7</strong></td>
<td><strong>Prolongs division phase</strong> of the cell cycle to promote replication. Responsible for <strong>malignant transformation</strong> of the cervical keratinocyte</td>
</tr>
</tbody>
</table>
Many women with positive test results from a HR HPV DNA Test will have normal biopsy. The HPV OncoTect™ E6, E7 mRNA Test is only positive if there is overexpression of E6, E7. In the life cycle of the HPV, the overexpression of E6, E7 mRNA in a cell is the molecular switch leading to cervical cancer.

The HPV virus invades the mucosa of the basal membrane of the cervix. From there the infected cell divides and spreads out in a lateral fashion. Some of the virus migrates to the suprabasal layers where viral genes are activated.
Virus particles assembled in terminally differentiated sq cells

Differentiated Cells. E & L viral genes expressed

Divided Cells (mitotic phase).
- Only E genes expressed
- Low levels of proteins formed

Virus infects an immature basal keratinocyte at low multiplication (<10 copies/cell). E1, E2, ?, E5, E6, E7

E5 binds to EGFR, PDGFR & CSFR & promotes cell proliferation but is frequently deleted from episomal viral DNA during integration into host genome.

Viral genome at thousands of multiples per cell. Leading to cell cycle arrest. E6, E7, E1, E2, E5

Viral DNA amplification in non-dividing cells.

Virus laden cells ready to desquamation and infection of naive individual L1, L2

6/12 wks

Baseline

HPVs & IMMUNE Response

- E6E7 Genes down-regulate interferon response
- Very low levels of proteins, no viremia
- No cell-death, no inflammation

In the absence of inflammation

- Keratinocytes release no pro-inflammatory cytokines
- No activation of LC and/ or no stromal dendritic cells
- No stimulus for DC activation, migration, antigen processing and presentation

Cytokines are crucial effectors in innate immunity and are released from dendritic cells after activation of receptors that recognise conserved structural motifs in pathogen specific molecules. Toll Like Receptors (TLRs) are an important family of receptors.

HR HPVs evade innate immune response and delay activation of adaptive immunity

Courtesy Dr Margaret Stanley, Dept of Pathology, Cambridge
L1 Gene targeting v/s E6E7 Gene Expression as a marker for risk assessment

- Currently available HPV DNA tests generally target conserved L1 region.

- The oncogenic effect is dependent on the E6 and E7 genes of the HR HPV types.

- Continuous expression seems to be necessary for transformation and maintenance of a neoplastic or dysplastic phenotype of the cells.

- Detection of E6/E7 transcripts of high-risk HPV types serves as a risk evaluation factor for the development of cervical neoplasia\(^1\).

- In addition, a repeated monitoring of a specific HPV type gives an indication of a persistent infection, which has been shown to be a predictor of high-grade lesions\(^2\).

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HPV life cycle is complex. During early stage of infection, viral DNA is present as a nuclear episomal form at a very low copy numbers in the basal cell layer of the stratified squamous epithelium. HPV DNA is amplified and encapsulated to progeny virions only in the terminally differentiated upper epithelial cells. Establishment of initial infection is directly linked to cell cycle progression through the mitotic phase.

Identifying integrated HPV DNA through this mitotic process helps identify CIN progression better than detecting free HPV DNA from the cervical exfoliative tissue.

Role of E6E7...

- HPV associated oropharyngeal cancer is a distinct entity
- HPV is a powerful prognostic factor for HNSCC
- Treatment modalities are under study currently for HPV +ve (and HPV –ve) disease
- Current research is focusing on local therapy, treatment intensity, the need for systemic therapy, and HPV biomarker refinement as the time is advancing
- HPV vaccination strategies to be addressed more precisely.

Expression of HPV E6/E7 oncogenes, the current gold standard for establishing a causal role for oncogenic HPV in human tumors.

Evolved HPV DNA Screening Procedure…

HPV mRNA Test: A Test for Future!

HPV DNA Tests

- Over-expressing E6, E7 genes that sets in cellular transformation
- Handling biological specimen without pre-treatment
- Differentiate cellular popln from TZ e.g. columnar and sq cells
- Possible to validate site specific SCC e.g. HNC, Anal, skin ca etc

Define your own choice!
THE HPV SPECIFICITY SPECTRUM

Screening studies comparing the specificity of various HPV tests to CIN2+ biopsy:


4. Weiss, et al, Intracellular HPV E6, E7 mRNA Quantification (HPV OncoTect) Predicts CIN 2+ in Cervical Biopsies Better than PAP Screening for Women Regardless of Age - Accepted Archives of Pathology and Laboratory Medicine 2011.
So... E6E7 mRNA POSITIONING

Main Companion Assay:

HC2 – molecular gold standard, proved its efficacy...

Positioned as an “adjunct” to primary test (VIA/Cyto/HC2 HPV in the Screening market and a confirmatory test in the Clinical Diagnostic market. Applied to following situations:

- NEWLY DIAGNOSED WITH ABNORMAL PAP SMEAR TEST
- REFLEX CONFIRMATORY TEST WITH LBC/PAP
- SUPPLEMENTAL CONFIRMATORY TEST FOR HC2 HPV
- COLPOSCOPY REFERRAL PATIENTS / ADJUNCT TO BIOPSY/HISTOPATH BEFORE CIN++ TREATMENT RECALL
- EPIDEMIOLOGICAL STUDIES & RESEARCH PROJECTS
Now...spend less time thinking about an *IDEAL* Cervical Screening test

CIN 3 histogram produced by OncoTect

and more analyzing it for clinical decision...