Bridging Morphology with Molecules

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Goals of this Lecture

The intent of this lecture is to discuss assays relevant to cytopathology and anatomic pathology, and illustrate how cytologists can integrate these skills into their clinical practice.
Presentation Outline

1. Introduction and Rationale
2. Hybrid Capture (Signal Amplification)
3. In situ hybridization (ISH/FISH) for nucleic acids
4. PCR
5. Invader Technology
6. Fluorescent ISH
7. Specific Oncology Probes
Technologist in Molecular Pathology, MP(ASCP)

- New Certification as of July 2003
- CTs and MTs are eligible without experience
- Test Breakdown:
  I. MOLECULAR SCIENCE (25-30%)
  II. MOLECULAR TECHNIQUES (35-40%)
  III. LABORATORY OPERATIONS (15-20%)
  IV. APPLICATIONS OF MOLECULAR TESTING (15-20%)

Complete content outline and suggested study material can be found on the ASCP website at www.ascp.org/bor
What’s So Great About Molecular Diagnostics?

- As many as 5,000 diseases have direct genetic causes
- High sensitivity and increased specificity for most tests adds diagnostic utility
- Potential for simple standardized procedures and automation
- Rapid throughput
- Increased number of techniques for infectious diseases and tumor diagnostics
- A viable reflex for equivocal morphology
  - Even one step further- DNA with Pap
- Prices are falling ↓
Why Perform Molecular Tests?

- Ability to Enhance Service/Patient Care
- Capacity to Enhance Profitability
- Avoiding Competition
- Convenience
- Retain Control of Testing Process
- Assure Testing Quality
- Underutilization of Technical Staff
- Integrate and Collect Data

Bolick, DR. Arch Pathol Lab Med, 2003;127:988
Current Molecular Techniques in Diagnostic Cytopathology

1. Hybrid Capture (HC)
2. In situ hybridization (ISH)
   A. Chromogenic
   B. Fluorescent
3. Polymerase Chain Reaction (PCR)
4. Invader Technology

Future Techniques (Investigational only)
1. Transcriptional Profiling (Microarrays)
   - FNA (Guidance of neoadjuvant therapy)
Digene Hybrid Capture®
DML 2000 System

Antibodies conjugated with thousands of enzymes attach to captured hybrids then react with a substrate to emit an amplified chemiluminescent signal.

1. Denature specimen
2. Specimen Hybridizes with RNA probe
3. Capture RNA probe detect hybrids on a solid phase
4. Luminescence
Liquid cytology, such as ThinPrep, facilitates Digene testing.
In Situ Hybridization

Morphology-Based Microscopic Diagnostics
What is *in situ* Hybridization?

Technique in which a single-stranded RNA or DNA probe is used to locate a gene or an mRNA molecule in a cell or a tissue.
The ISH Staining Process

Alkaline Phosphatase conjugated Avidin

Fluorescein-Labeled probe

Biotinylated linking Antibody

Primary antibody: anti-FITC, anti-Biotin or Anti-Digoxigenin

Target

FISH (Fluorescent in situ Hybridization)

Bright Field (Chromogenic detection)
Denaturation and Hybridization

- Increasing temperature to denature and separate target nucleic acid strands
- Addition of labeled probe
- Reducing temperature to hybridize probe to target
- Post hybridization stringency washes
Polymerase Chain Reaction

http://www.unlv.edu/staff/wmojica/PCR_LAB2.htm
Invader® Technology (Third Wave, Inc.)

- Directly detects specific nucleic acid sequences
- Based on signal amplification
- Isothermal reactions; no thermal cycling
- Method
  - An overlapping structure created with the mutant probe and the Invader® oligo. Cleavase® enzymes cleave the primary probes that form overlapping structures releasing the 5' flaps plus one nucleotide.
  - Released flaps from the primary reaction serve as oligos in a second simultaneous reaction on a labeled, synthetic oligo (Fluorescence resonance energy transfer (FRET) probe).
  - Cleavage of this FRET probe = fluorescent signal.
- Produce 1 million to 10 million labeled cleavage products per target sequence
Invader® Technology

Invader® Oligo

Probe

Genotype specific target

Released 5´ Flap

Cleavage Site

FRET Cassette 1

F1

F2

Invader® Oligo

Probe

Control target

Released 5´ Flap

Cleavage Site

FRET Cassette 2

F1

F2
Applications of Molecular Diagnostics

A. Infectious Disease Markers
   1. HPV
   2. CT/GC
   3. CMV

B. Oncology Markers
   a. Her2/neu
   b. Urovision, LaVision, Provision, Pathvision
   c. Kappa/Lambda
   d. CK20, t(8;21), t(9;22), t(12;18), t(15;17)
   e. BCRA 1 and 2
   f. FNA applications
Molecular Techniques for HPV Detection

- It is the second most common cancer among women worldwide
- 500,000 women, usually in their 30s or 40s, are stricken by the disease annually (Central Am, Africa, Asia)
- Estimated that by 2020 more than 1 million new cases will emerge each year
- About 266,000 women in the Asian-Pacific region--more than half of the total number of women afflicted worldwide - and 143,000 die each year
Current HPV DNA Test Techniques

- Hybrid Capture 2 (HC2)
- In Situ Hybridization (ISH)
- Invader Technology
- Polymerase Chain Reaction (PCR)
HPV DNA Testing Uses

- Triage of Atypical squamous cells
- Primary Screening Co-test with Cytology
- Quality assurance and clinical follow-up:
  - Monitor percent HPV positive by category
  - Additional tool with cytology as “test of cure” in clinical follow-up and as basis for referral to diagnostic excisional procedures (conization, leep, etc)
Hybrid Capture Method

- Performance Characteristics
  - 83-96% Sensitivity in CIN 2+
  - 98% Negative Predictive Value
  - 61% Specificity
  - 17.2% Positive Predictive Value

Data is from Digene package insert and JAMA Bethesda Guidelines
HC2 HPV DNA Testing: Pros and Cons

- Only FDA-approved HPV DNA test option
- Only HPV DNA test option with supporting Class I clinical evidence of ALTS used to formulate clinical follow-up guidelines
- Clinical sensitivity of 85-100% for HSIL and cancer
- Specificity issues with false-positives and cross-reactivity to other HPV types
- Does not differentiate between HR and IR viral types or genotype
- Limited data establishing effectiveness in detection of AIS and cervical adenocarcinoma, now representing around 1/3 of new US cervical cancers and most litigated cervical cancer cases
Ventana ISH Testing Method

- Automated in situ hybridization (ISH) assay
- Analyte Specific Reagent (ASR) for High and Low Risk types of HPV
- Can be performed on Liquid Based Pap and Tissue Biopsies
- Chromogenic Assay
In Situ Hybridization for HPV: Pros and Con’s

- Excellent tissue or cytology localization of HPV-infected cells
- Good specificity (>95%)
- Fully automated with walk-away capacity
- Differentiates between episomal (diffused) versus integrated (punctate) HPV DNA patterns
- ASR labeling places an additional responsibility on the medical director to assure performance characteristics
- No data on prognostic value for future disease
- Higher cost for payers and patients (could be addressed if studies establish method as reliable but more selective basis for colposcopic referral)
**Invader® Technology**

- **Invader® Oligo**
- **Probe**
- **Genotype specific target**
- **Released 5’ Flap**
- **Cleavage Site**
- **FRET Cassette 1**
- **FRET Cassette 2**

Invader® Technology involves the use of specific oligonucleotides (Invader® Oligo) and probes to cleave and release 5’ flaps, generating FRET signals. The technology is used for genotyping specific targets and control targets. The FRET signals are generated by the cleavage of the FRET cassettes.
Invader® Technology: Pros and Cons

- **Ease of Use**
  - Minimal start up costs and no specialized training
  - Simple procedure enhances throughput and cost-efficiency
  - Consistent product format
  - Straightforward, objective results
- **Very limited data to date**
- **Sensitivity for CIN 2/3 and cancers not yet established in published studies**
- **ASR labeling**
- **No data on prognostic value for future disease**
- **Use of some molecular CPT codes may need to be negotiated with payers by the laboratory**
Polymerase Chain Reaction (PCR)
PCR: Commercial tests for the future?

- Commercially available test versus a “home brew” test?

- Home Brew
  - Typing done with Sequencing (Blastn) or Dot/Line Blot hybridization
  - Extremely sensitive - as few as 10 copies
  - Can be performed using Parrafin-embedded cervical tissue or liquid based Paps
  - Works beautifully with TP specimens
PCR - Gel Electrophoresis
Pilot Study of a Commercialized Human Papillomavirus (HPV) Genotyping Assay: Comparison of HPV Risk Group to Cytology and Histology

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Received 16 June 2005/Returned for modification 5 August 2005/Accepted 30 August 2006

We evaluated a commercialized PCR assay, Linear Array, that detects 37 HPV genotypes, using a sample of liquid cytology specimens (n = 534). We found a strong association of an increasing level of HPV risk (HPV type 16 [HPV16] > HPV18 > other carcinogenic types > noncarcinogenic types > negative specimens) with increasing severity of cytologic interpretations (P = 0.0002) and histologic diagnoses (P = 0.00005).

Carcinogenic human papillomaviruses (HPV) testing has now been approved in the United States as an adjunct to cytology for triage of equivocal cytology at all ages and for general screening for women of ≥30 years old (15). Several studies have now shown that detection of specific carcinogenic HPV types, especially HPV type 16 (HPV16) and HPV18, may be useful in differentiating carcinogenic HPV-positive women at greater and lower risk of having or developing precancer, cervical intraepithelial neoplasia grade ≥3 (CIN3), or cancer (CIN7+). (1, 2, 8). Identifying women with persistent carcinogenic HPV infections may also be clinically useful (9, 10). Together, these data support a role for HPV genotyping in cervical cancer screening.

Commercial HPV genotyping assays are currently under development. We evaluated one assay, Linear Array (LA; Roche Molecular Systems, Alameda, CA), a PCR-based genotyping assay that detects 37 HPV genotypes which is a commercialized version of the Linbro assay (11).

MATERIALS AND METHODS

Cervical specimens and tests. We acquired blinded residual CancerNet specimens, after optoeptic interpretation had been rendered, from 534 women with normal smears, 155 women with cervical intraepithelial neoplasia grade 2 (CIN2) and/or 3 (CIN3) from the National Cancer Institute Surveillance, Epidemiology, and End Results Program (SEER), and 155 women with grade 2 and 3 cervical intraepithelial neoplasia grade 3 (CIN3+) (n = 155) from the National Cancer Institute Surveillance, Epidemiology, and End Results Program (SEER). The institutional review board (IRB) of the Medical University of South Carolina approved the study, and the use of these specimens was deemed exempt from review by the UC IRB. We subsequently excluded 13 specimens of CIN3+ because of contamination and 1 specimen from a known control of cervical abnormalities (e.g., intestinal metaplasia).

In addition to the original histologic diagnosis, each specimen underwent a manual re-review to assess the histologic diagnosis. Importantly, all cases originally coded CIN3+ and one case coded CIN6 were reclassified as CIN2, and eight cases originally coded CIN5+ and one case coded CIN6 were reclassified as CIN2. Also, two cases coded CIN2 and two cases coded CIN2- were reclassified as CIN1. We then used our own history to select cases.

RESULTS

We found a strong trend of increasing severity of cytologic interpretation with increasing likelihoods of testing positive by LA for any HPV type (P = 0.0016) and of testing positive
HPV Genotyping Assays

- **Roche, Inc.**
  - Linear Array
- Evaluated liquid cytology specimens (n = 534) and found a strong association with HPV risk group (HPV16 and HPV18 with increasing severity of cytology (PTrend < 0.0005) and histology (PTrend < 0.0005)).

HPV Genotyping Assays (TaqMan)

Roche, Inc.

COBAS TaqMan PCR Genotyping Assay

- **Purpose:** Identify presence of 16 carcinogenic HPV types in aggregate while concurrently providing HPV16 and HPV18 genotype information from cervical specimens.

- **Results:** For detection of HPV16 and 18, there was 98.0 and 99.0% agreement between TaqMan HPV testing Linear Array genotyping.

*Sadorma M, Castle PE, Garcia FR, Holladay EB, Kornegay J. Ability of a prototype COBAS TaqMan HPV test to simultaneously identify high risk HPV infection and provide HPV16 and HPV18 genotyping. International Papillomavirus Conference. Prague, Czech Republic. June 2006.*
HPV Genotyping Assays (APTIMA)

• GenProbe Inc.

APTIMA® Human Papillomavirus (HPV) Assay

• HPV mRNA for E6 and E7 oncoproteins may be a more specific biomarker for cervical precancer and cancer than carcinogenic HPV DNA

• Method: detected oncogenes from E6/E7 mRNA for 14 carcinogenic HPV genotypes on liquid cytology specimens (n = 531) to the presence of HPV genotypes detected by PGMY09/11 L1 consensus primer PCR assay

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Abstract

Purpose: To evaluate carcinogenic human papillomavirus (HPV) E6/E7 mRNA for E6 and E7 mRNA detection on clinical specimens to identify women with cervical precancer and cancer.

Experimental Design: We evaluated a prototype assay that collectively detects oncogenes E6/E7 mRNA for 14 carcinogenic HPV genotypes on a sample of liquid cytology specimens (n = 531), masked to clinical data and to the presence of HPV genotypes detected by PGM09/11L1 consensus primer PCR assay.

Results: We found an increasing likelihood of testing positive for carcinogenic HPV E6/E7 mRNA with increasing severity of cytology (P = 0.00001) and histology (P = 0.00001), with 94% of cervical intraepithelial neoplasia grade 3 (CIN3) histology cases (46 of 49) and all five cancer cases testing positive for carcinogenic HPV E6/E7 mRNA. Overall, fewer specimens tested positive for carcinogenic HPV E6/E7 mRNA than for carcinogenic HPV DNA (P = 0.0001, McNemar's χ² test), especially in women with CIN3 (P = 0.0001). We also found that using a higher positive cutoff for detection of carcinogenic HPV E6/E7 mRNA improved the association of positive test results with cervical precancer and cancer by reducing the number of test positives in women without precancer without reducing clinical sensitivity for cervical precancer and cancer compared with detection of carcinogenic HPV E6/E7 mRNA using a lower positive cutoff by the same assay and with detection of carcinogenic HPV DNA.

Conclusions: We found that carcinogenic HPV E6/E7 mRNA is a potentially useful biomarker for detection of cervical precancer and cancer and warrants further evaluation.

Cervical infections by ~15 carcinogenic HPV types cause virtually all cervical cancer and the immediate precursor (precancerous) lesion, cervical intraepithelial neoplasia grade 3 (CIN3; also known as carcinoma in situ [CIS]; refs. 1–3). Persistence of carcinogenic HPV is necessary for the development of cervical precancer and cancer (4); detection of carcinogenic HPV type-specific persistence strongly predicts the development of precancer and cancer (5, 6). Diagnoses of CIN3 or frank cancer require treatment; CIN2, a less certain diagnosis of cervical precancer (7), is also treated as a margin of safety.

Based on knowledge of the central role for persistent carcinogenic HPV in cervical carcinogenesis, one pooled probe test for the carcinogenic types of HPV DNA has already been Food and Drug Administration (FDA) approved, and other tests will soon be widely available. HPV DNA negativity is associated with an extremely low risk of prevalent or incident CIN3 or cancer (≥CIN3; refs. 8, 9). Carcinogenic HPV testing has now been approved in the United States as an adjunct to cytology for triage of equivocal cytology at all ages and for general screening in women ≥30 years old (10). There is now significant evidence that carcinogenic HPV DNA detection is significantly more sensitive but slightly less specific for detection of cervical precancer and cancer than cytology (11). Currently, there are no FDA-approved tests for detection of specific HPV genotypes, which could be used to measure persistent carcinogenic HPV infection, although repeatedly testing positive for carcinogenic HPV types in aggregate confers an elevated risk of precancer (12) considerably as surrogate for type-specific persistence.

During normal productive HPV infections, the expression of viral genes is tightly regulated and linked to the differentiation state of the epithelium. At the basal layer of the epithelium, where HPV infection is established, the viral genome is maintained. Early proteins, such as oncoproteins E6 and E7, are expressed at low levels for genome maintenance and cell proliferation. As the basal epithelial cells differentiate, the viral...
HPV Genotyping Assays (APTIMA)

• Results:
  • An increasing likelihood of testing positive for carcinogenic HPV E6/E7 mRNA with increasing severity of cytology (PTrend <0.0005) and histology (PTrend < 0.0005), with 94% of CIN3 histology cases (n = 49) and all five cancer cases testing positive for carcinogenic HPV E6/E7 mRNA
  
  • Fewer specimens tested positive for carcinogenic HPV E6/E7 mRNA than for carcinogenic HPV DNA ((P < 0.0005, McNemar’s c2).

HPV Testing

- **Take home message:**
  - Specificity and sensitivity is clearly improving for HPV genotyping
  - Roche has submitted LA and Amplicor for FDA approval as of March 5, 2007
Cancer and Molecular Dx

Cancer is a multi-step genetic disease, resulting from specific alterations in the function of one or more genes.

- Molecular changes occur at the chromosome, gene & sequence level.
- Important to detect the full range of genetic abnormalities that are indicative of cancer.
  - ISH and FISH which detects chromosome copy number.
  - PCR and FISH which can detect gene expression.
  - Sequencing for detection at the base pair level.

![Diagram of a normal cell, chromosomes, genes, and DNA sequences.](image)
Trends in Disease Management

- Individual genetic composition will determine therapeutic selection
- Will need to interpret increasingly complex data sets
  - Simultaneous evaluation of multiple genetic targets
  - Integration of genetic data with therapeutic effectiveness

Genomic Assessment

Bioinformatic Data Reduction for Clinical Utility

Guided Therapeutics Disease Prevention

Disease Prevention

Bioinformatic Data Reduction for Clinical Utility

Guided Therapeutics Disease Prevention
Trends in Disease Management

- Pharmacogenomics a key driver — genetic variations that influence the response to therapeutic drugs.
Oncology Markers

- Used to assess genetic mutations for an individual patient’s tumor (a.k.a. tumor profiling)
- Helps guide clinicians in selecting the most appropriate therapeutic regimen (for instance, N-myc amplification in neuroblastoma as an indicator of cisplatin resistance)
- Development of molecular pharmacogenetics is results in new prognostic indicators being used for the assessment of individual tumors
Oncology Markers

a. Kappa/Lambda
b. FISH (Bladder, Lung, Breast) Urovision, LaVision, Pathvision
c. Her2/neu
d. BCRA 1 and 2
e. Other
   a. p53
   b. p16
c. K-RAS
Kappa and Lambda for Lymphoma

- Detection of Kappa and Lambda light chain mRNA in plasma cells and B-lymphocytes
- Each immunoglobulin molecule contains either two
- Copies of Kappa or lambda light chains
  - K/L ratio 2:1 = Reactive Lymphoid Hyperplasia
  - K/L or L/K ratio 3:1 or greater: B cell Lymphoma *

[Image of lymphocyte staining]
Urothelial Carcinoma

- 340,000 cases/year
- 130,000 deaths
- >95% of the bladder are Urothelial Carcinomas in Europe, North America, Australia
- Bilharzial bladder cancer (squamous cell carcinoma)- most common in Northern Africa
Sensitivity of cytology is good for establishing the diagnosis of high-grade urothelial tumors, low-grade tumors are difficult and often impossible to distinguish from benign urothelial cells.

Most bladder cancer recurs, and early recurrence is often difficult to detect cytologically with cystoscopic specimens.
Laboratory Detection of UC

- *Antigen based methods
  BTA-Stat, Immunocyt, NMP22, FDP
- Cytology
- Molecular genetic methods
  Telomerase, microsatellite analysis
- Flow cytometry/Digital Image Analysis
- Cytogenetic methods
  FISH (Vysis, Inc. (UroVision)) multi-color test that detects aneuploidy for chromosomes 3, 7, 9p21, and 17

*not shown utility for confirming the primary diagnosis of low-grade urothelial neoplasms.
UroVysion Assay Overview

- Chromosome 3: CEP 3 (SpectrumRed)
- Chromosome 7: CEP 7 (SpectrumGreen)
- Chromosome 9: LSI 9p21 (SpectrumGold)
- Chromosome 17: CEP 17 (SpectrumAqua)

NORMAL

BLADDER CANCER
Fine Needle Aspiration Biopsy (Applications)

- Growing trend in FNA cytology to better classify genetic mutations that can aid the cytologist in stratifying aggressive versus less aggressive lesions.
  
  - a. Neuroblastoma: N-Myc characterization using fluorescent in situ hybridization (FISH)
  
  - b. Thyroid (papillary) cancer:
    - the RET tyrosine kinase domain (RETTK) using PCR
    - BRAF mutations by PCR; preoperative dx for PTC; not seen in benign nodules
    - Telomerase activity: limited use due to + thyroiditis
  
  - C. Ewings sarcoma: FISH; t(11:22) (q24;q12) chromosomal translocation using the SLI-EWSRI (22q12) dual color probe
Breast Cancer: Her2/Neu

• 25% of breast cancer patients have tumors which overexpress Her2/neu gene (chromosome 17 (17q11.2-q12)

• ER/PR negativity and a poor prognosis. Patients are eligible for Herceptin (trastuzumab) therapy

• Immunohistochemistry (IHC) or protein overexpression for Her2/neu often is difficult to discern (due to weak positive signals or background staining)

  • Large percentage of tumors considered positive by IHC (>=2) for Her2/neu by IHC show no gene amplification (presence of detectable mRNA); therefore, ICH was falsely positive.
Differences in Interpretation: HER-2 Assessment by FISH and IHC

- **3+**: High amplification
- **2+**: Low amplification
- **1+**: Normal
- **Negative/0**: Normal

Images show varying levels of HER-2 protein expression and gene amplification.
Her2/neu

- FISH testing may have a sensitivity and specificity of 96-100% for assessing over expression of the Her2/neu gene.
- Enhances the opportunity for more effective therapeutic triage.
  - Vysis, Inc. (PathVision)
    - (ratio of Her2/neu to CEP 17) prevents misdiagnosis in cases with polysomy for chromosome 17.
  - Oncor, Ventana (ISH)
    - Her2/neu only
  - * Ventana Benchmark
    - Her2/neu only
  * Not FDA approved
FNA Applications

- **Breast cancer:**
  - Human androgen receptor monoclonality has been linked to breast cancer and reflex testing for equivocal or “atypical” breast aspirates (as well as ductal lavage)
  - Loss of heterozygosity (LOH) of p53 oncogene mutations are found in 50% of all human cancers, including up to 80% of breast cancer (17p13.1) and colon cancer
FNA Applications

- **Pulmonary Neoplasms**
  - Rationale: differentiation between “atypical”, “suspicious”, or “indeterminate”
  - CNB could not be performed or because of the development of pneumothorax
  - LaVision (Vysis) multitarget FISH assay for 6p11-q11, 7p12 (EGFR), 8q24 (myc), and 5p15.2-chromosomal loci affected in non-small cell lung carcinoma

- **Renal**
  - RT-PCR of MN/CA9 gene expression response to hypoxic conditions
    - Clear RCC (100%) and papillary RCC (56%). Not found in chromophobe RCC, oncocytoma, or normal tissue
FNA Applications

- Pancreatic cancer:
  - Paucity of diagnostic material, overlapping features of low grade malignancies, chronic pancreatitis
  - K-\textit{ras} mutations are seen in 90% of pancreatic carcinomas and up to 80% of cholangiocarcinomas
  - Teleomerase activity: limited use: not expressed in all PN

- Effusions: FISH analysis for chromosomes 3, 8, 10, and 12 are being used to determine hyperdiploidy for equivocal or “atypical” cells
FNA Applications

- Metastatic Tumors in lymph nodes
  - Micrometastasis in EUS-FNAB to r/o gastrointestinal vs lung: hypermethylation of CpP islands in promoter regions of MGMT, p16 and p14

- Infectious disease:
  - HPV: differentiate anogenital or head and neck SCC versus esophagus, lung and skin SCC
Future FNA Applications

Colon Cancer

- Hereditary nonpolyposis colorectal cancer, an inherited syndrome and analysis of mutations associated with familial polyposis (FAP) and attenuated FAP is available with COLARIS ApSM.
  - analysis is a PCR-based assay (Myriad Genetics).

Melanoma and Pancreatic Cancer: the p16 gene mutation can be determined with PCR using MELARISSM (Myriad Genetics).
New Technologies: Not all Molecular
P16 as a Marker for Cervical Cancer?

- Overexpression of p16INK4a protein indicates infection and genomic integration of high-risk human papillomavirus (HR HPV) and predicts progression to cervical high-grade squamous intraepithelial lesions (HSILs) and carcinoma.

- p16INK4a protein was immunolocalized using a specific monoclonal antibody, and the detection of HR HPV in all 400 specimens was determined using HC2.

- p16INK4a was found to be positive in only 78% of HSIL

p16 as a Screening Test

- HSIL-3118
  HPV DNA positive
  p16 true positive

- HSIL-3110
  HPV DNA positive, p16 true positive

p16 as a Screening Test

WNL-3027
HPV DNA negative, p16 false positive metaplastic cell

HSIL-3127
HPV DNA positive, p16 false negative

Mutiplexing

- **Luminex, Inc.**

  - Color-codes tiny microspheres (100 distinct sets) coated with a reagent specific to a particular bioassay, allowing the capture and detection of specific analytes from a sample.

  - Analyzer lasers excite the internal dyes that identify each microsphere particle allowing multiplexing of up to 100 unique assays within a single sample - reducing cost and labor

  - Wide variety of applications - protein expression profiling (cancer biomarkers AFP, CEA, PSA and molecular infectious disease (HPV, HIV, HCV, HBV)

  - Open architecture platform allows other companies to provide compatible kits
The Future of Molecular Pathology

Glass slide microarray of thousands of genes for evaluation as new biomarkers for cancer prognostic assessment

Eleftherios P. Diamandis, M.D., Ph.D., FRCP(C)
Mt. Sinai Hospital and Univ. of Toronto
Genome: 12,800 points
Diameter: 120 microns
Slide size: 170 mm x 340 mm

Eleftherios P. Diamandis, M.D., Ph.D., FRCP(C)
Prognostic Signature of Breast Cancer

Patients **above** line:  
No Distant Metastasis

Patients **below** line:  
Distant metastasis

*Van’t Veer et al. Nature; 415:530-536*
The Future??

Cancer Patient
↓ Surgery/Biopsy

Cancerous Tissue
↓ Array Analysis

Tumor Fingerprint
↓ Individualized Treatment

Eleftherios P. Diamandis, M.D.,