

LABletter

PATHOLOGY & LABORATORY MEDICINE NEWSLETTER

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Increasing Positive Predictive Value for Pre-Cervical Cancer Lesions with HRDNA and HPV E6, E7 mRNA

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- Cervical cancer continues to be a major women's health issue even with significant milestones in detection & prevention
- High risk HPV DNA (HRHPV) detection part of the cervical cancer screening algorithm over the past decade
- HPV OncoTect increases the positive predictive value of cervical cancer from 15-25% with HRDNA alone to >90% using a combination of HRDNA and HPV E6, E7 mRNA

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The Role of Prealbumin as a Marker for Nutritional Status

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Increasing Positive Predictive Value for Pre-Cervical Cancer Lesions... with HRDNA and HPV E6, E7 mRNA

Cervical cancer is the second most common cancer among women worldwide.

According to National Cancer Institute estimates, approximately 11,150 new cases of cervical cancer will be diagnosed in the United States in 2007 with 3,670 deaths.

Cervical cancer continues to be a major women's health issue even though significant milestones have been reached in cervical cancer screening that include:

- Conventional Pap, in wide use since 1940s,
- Discovery of liquid-based cytology in mid-90s,
- Revised screening guidelines (The Bethesda System) that came into effect in the early 2000s, and
- Vaccines launched in 2006

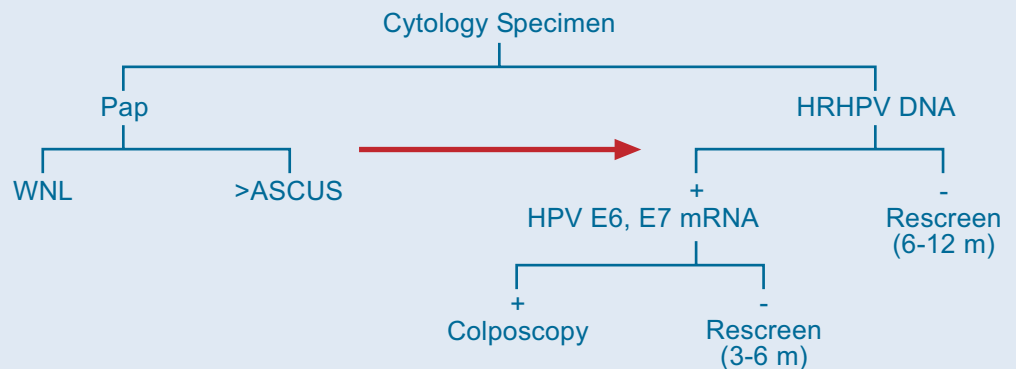
High risk HPV DNA (HRHPV) detection has become part of the cervical cancer screening algorithm over the past decade.

Initially approved by the FDA for cytologic samples diagnoses as atypical squamous cells of undetermined significance (ASCUS), HPV is now approved for all cervical cytology specimens and a patient management algorithm (Fig.1) has been developed based on the cytology test result and the presence or absence of high risk HPV DNA.

Herein lies the problem. High risk HPV DNA is **NOT** a test for women that have a pre-cancerous or cancerous lesion of the cervix as indicated by a cervical biopsy diagnosis of cervical intraepithelial neoplasia (CIN) 2,3 or invasive cancer.

HRHPV indicates that women have a RISK of developing cervical cancer. This risk for HPV 16, the most virulent of HPV types is 4.4%. HRHPV DNA-based HPV tests have a very high negative

Fig.1 HPV/Cervical Cancer Screening Algorithm Using HPV E6, E7 mRNA to Improve Positive Predictive Value



From Irwin D. Clinical Virology Symposium, Clearwater Beach, FL May 3, 2006

“HPV OncoTect increases the positive predictive value of cervical cancer from 15-25% with HRDNA alone to >90% using a combination of HRDNA and HPV E6, E7 mRNA.”

predictive value (~99%), which can allow for longer screening intervals, however, the positive predictive value of these tests is 15-25%. This translates into a high false positivity rate and many women sent for unnecessary colposcopy and biopsy.

In a recent article by Dr. Eileen Burd, Chief of Microbiology at Emory University in Atlanta, she described the use of HPV OncoTect, a diagnostic test for the HPV oncogenes E6, E7. The presence of high levels of HPV E6, E7 mRNA in individual cells indicates that these cells are undergoing the molecular changes of cellular transformation that are the early stages of cervical cancer development.

The HPV OncoTect technology (Fig.2 & 3) is being used at Stanford and has been shown to increase the positive predictive value of cervical cancer screening from 15-25% using HRDNA alone to >90% using a combination of HRDNA and HPV E6, E7 mRNA. Ultimately, this determines which women have a precancerous lesion and which women really need colposcopy and biopsy.

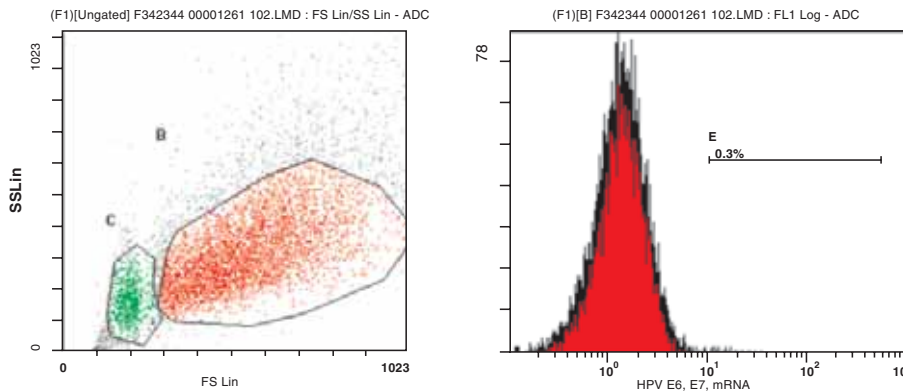
Ongoing research studies with this test in Spain, have shown that HPV E6, E7 mRNA helps determine which women with CIN 1, a very mild form of dysplasia that usually clears on its own, will progress to more advanced disease.

More about E6-67: HPV OncoTect™ is the first test that quantifies E6 and E7 mRNA, the genes associated with cervical cancer. Unlike PCR which destroys cells during the process, HPV OncoTect™ works in a reagent platform that performs molecular biology in the cells and identifies which cells are making the genes that cause cancer.

The HPV OncoTect Assay identifies HPV E6, E7 mRNA via in situ hybridization with oligonucleotides specific for HPV E6, E7 mRNA and quantitates the overexpression of HPV E6, E7 mRNA via flow cytometry. The HPV OncoTect Assay can be carried out in less than three hours directly from liquid-based cervical (“LBC”) cytology specimens.

Fig.2

HPV ONCOTECT (STANFORD) Within Normal Limits



See: www.stanfordlab.com under publications for the previously published article by Dr. Bruce Patterson, “Recent Advances in Cervical Cancer Screening” in the *SUMCCL Pathology & Laboratory Medicine LabLetter*, June 2006, Volume 2, Issue 3 or contact your SUMCCL sales representative for a copy.

References:

1. Roberto Narimatsu, MD, and Bruce K. Patterson, MD, B.K. 2006. High-Throughput Cervical Cancer Screening Using Intracellular Human Papillomavirus E6 and E7 mRNA Quantification by Flow Cytometry. *Am J Clin Pathol* 2005;123:716-723*

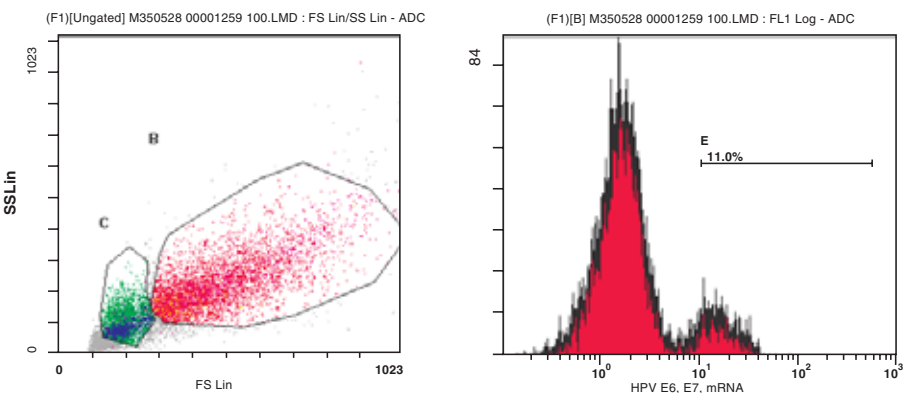
2. Invirion Diagnostics, LLC BUSINESS WIRE 3/13/2007. www.invirion.com.

3. Grundhoefer, D. and B. K. Patterson. 2001. Determination of liquid-based cervical cytology specimen adequacy using cellular light scatter and flow cytometry. *Cytometry* 46:340-344

*Please address reprint requests of this published article to:
labmarketing@stanfordmed.org.

Fig.3

HPV ONCOTECT (STANFORD) CIN 3 - Carcinoma-in-situ



Laboratory Update:

Urgent Communication

Transport Policy for Outreach CSF Specimens

- Stat Transport Available for Outreach STAT CSF Testing
- Monday – Friday 9:00 AM - 5:00 PM

Please call for a STAT pick-up to the laboratory **immediately after collection**. Cell lysis can begin within one hour of collection. Place in a RED STAT* Biohazard Specimen Transport bag and follow temperature transport requirements.

Be sure to note Telephone number where you can be reached with STAT results.

- Specimens will be delivered to Stanford Clinical Lab at Hillview for immediate analysis. **Hand directly** to the laboratory personnel (courier) (do not leave on counter or lock-box).
- The majority of CSF analysis requires immediate Refrigeration or Freezing. Exceptions that are transported at Room Temperature are Cultures, Gram Stain & Cryptococcus Antigen.

*available from the Supply Department

CELL COUNT AND CHEMISTRY ANALYSIS

Cell Count & Differential

Order Code: CSFCT

Volume: 2 mL (1mL min), Refrigerated

Glucose

Order Code: CSFGL

Volume: 1mL min, Freeze immediately

IgG/Albumin Ratio CSF

Order Code: YCIGG

Volume: 2 mL (1mL min), Freeze immediately

Other CSF Analysis

See Test Directory for specific Handling and Transport Instructions or call Customer Service at 1-877-717-3733 for information.

FISH CHIC2

Order Code: CGFi CHIC2

Synonyms: FISH, FIP1L1/PDGFR A gene rearrangement; FIP1L1; PDGFR A; deletion 4q12; del(4q12)

Specimen Type: Whole blood, bone marrow, or other cellular fluid

Container Type: Green-top tube (sodium heparin), or sterile container for other fluid

Required Volume: 2 mL

Minimum Volume (Ped): 1 mL

Methodology: Fluorescence in situ hybridization, deletion probes, interphase analysis

Performed: Mon-Fri

Reported: 12-14 days

Special Handling: Transport at room temperature.

CPT Codes: 88271 x 3, 88275

Cytogenetics: New Assays

- Array Comparative Genomic Hybridization (aCGH)
- FISH CHIC2

General Test Information and Clinical Indication:

Array Comparative Genomic Hybridization (aCGH): Array comparative genomic hybridization (aCGH) is a new method using existing array technology to detect some chromosomal abnormalities that are too small to be appreciated by high-resolution chromosomal analysis. Typically, high-resolution chromosomal analysis can detect chromosomal abnormalities of between 5 to 10 Mb in size. Depending upon the type of array and the spacing of the features on the array, the size of detectable anomalies can be anywhere from a few hundred kilobases of DNA to less than 5 Mb.

Our method utilizes a microarray carrying 44,000 oligonucleotide (Agilent) sequences from known genomic locations at an average spatial resolution of 35 kb of DNA. Patient and control DNA samples are labeled using different fluorescent tags. These DNA samples are then hybridized to the array. The arrays are scanned and the intensities of the fluorescence are plotted as a ratio at each oligonucleotide probe. These ratios can then be interpreted as representing either a loss or a gain of DNA. A copy number change is considered only when three or more adjacent oligonucleotides are shown to be duplicated or deleted (with an average size of ~ 100 kb).

Please note: This array cannot detect polyploidy, balanced chromosomal rearrangements; mosaicism, small duplications or deletions below the resolution of this array or point mutations.

Array-based Comparative Genome Hybridization, Genetic Dx.

Order Code: CGH GEN

Synonyms: aCGH, CGH, array CGH

Specimen Type: peripheral blood

Container Type: Green-top tube (sodium heparin)

Required Volume: 10 mL

Minimum Volume (Ped): 2 mL

Methodology: array-based comparative genome hybridization

Performed: Mon, Wed

Reported: 14 days

Special Handling: room temperature; blood specimens of both parents also required

CPT Codes: 88386 x 4, 83891, 83892 x 2

Causes For Rejection: Improperly labeled specimen, incorrect specimen, hemolyzed or coagulated specimen

FISH CHIC2: CHIC2 Deletion (or FIP1L1/PDGFR A fusion): Interphase fluorescence in situ hybridization (FISH) is performed using a set of differentially labeled probes specific for three different loci – CHIC2, FIP1L1 and PDGFR A. Loss of the CHIC2 probe is used as a surrogate for fusion of FIP1L1 and PDGFR A. CHIC2 deletion (or FIP1L1/PDGFR A fusion) can be observed in individuals with hypereosinophilic syndrome (HES) or systemic mast cell disease (SMCD). The FIP1L1/PDGFR A fusion creates a novel tyrosine kinase which can be a therapeutic target for imatinib mesylate (or Gleevec).

Q&A: Submit Q&A topics of interest to: labmarketing@stanfordmed.org

An opportunity for the Bay Area Medical Community to request specific Q&A topics to our medical directors that are relevant to patients' clinical needs.



Raffick Bowen, Ph.D.

The Role of Prealbumin as a Marker for Nutritional Status

Q&A with Raffick Bowen, Ph.D., Associate Director of Chemistry & Immunology and Assistant Professor of Pathology.

Q1: Why are serum markers of protein nutrition needed?

A1: Although severe defects in protein metabolism (such as marasmus or kwashiorkor) are unusual in this country, many patients may be suffering from a negative nitrogen balance and could benefit from nutritional support. These include patients undergoing surgery, hemodialysis, trauma, treatment of infection or chronic wound care, as well as many elderly patients whose reserve of protein may be decreased. Intervention may decrease morbidity and mortality; decrease medical and pharmaceutical costs; and decrease length-of-stay. Protein malnutrition occurs before there is any significant weight loss. Although accurate nutritional assessment tools like anthropometric measurements have been developed, they are time-consuming, require skilled investigators, and are not suited to large-scale use. Another alternative is the measurement of a marker of protein metabolism in the clinical laboratory.

Q2. What is prealbumin?

A2: Prealbumin is a protein that, like albumin, is synthesized by the liver. It has two major functions: transport of thyroxine and vitamin A (via retinol-binding protein). For these reasons, it is also known as transthyretin. Prealbumin has a high concentration of tryptophan, which has been shown to play a key role in the initiation of protein synthesis. Prealbumin has a relatively short half-life in the circulation, compared to albumin, and its synthetic rate is very sensitive to intake of adequate nutrition. Therefore, quantitation of serum prealbumin has a major clinical utility as a marker for nutritional status.

Q3: What are “normal” levels of prealbumin?

A3: A normal prealbumin level is 20-40 mg/dL using a nephelometric analyzer. Serum prealbumin concentrations between 10-20 mg/dL are considered below normal, indicating increased nutritional risk. Nutritional risk is significant when prealbumin level is between 5 and 10 mg/dL; a poor outcome is predicted when level of less than 5 mg/dL is obtained.

Q4: Who should be tested for prealbumin?

A4: A consensus exists in the nutritional community that prealbumin should be measured in all patients admitted to hospital at risk of undiagnosed negative nitrogen balance. These include elderly patients and patients with diabetes and/or renal disease. In order to make this test more available, we are bringing it in-house this month. Although the evidence that measuring prealbumin may be useful in the out-patient setting is less plentiful, physicians may also wish to consider ordering this test in certain populations, such as patients on hemodialysis.

Q5: What are some of the limitations of prealbumin testing?

A5: Like most laboratory tests, prealbumin is not perfect. Like albumin, prealbumin is a negative acute phase reactant and levels will decrease in the presence of inflammation and the immediate post-surgical period even in patients with adequate protein nutrition. Furthermore, prealbumin levels will be decreased in zinc but not vitamin deficiency. Also, prealbumin should not be considered as a substitute for a comprehensive nutritional assessment. Rather, it can help identify those patients who may need closer scrutiny.



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