Molecular Approaches to Cervical Cancer Screening
HPV and Beyond

New Horizons in Molecular Technology
Asia Symposium on Advanced Molecular Technologies

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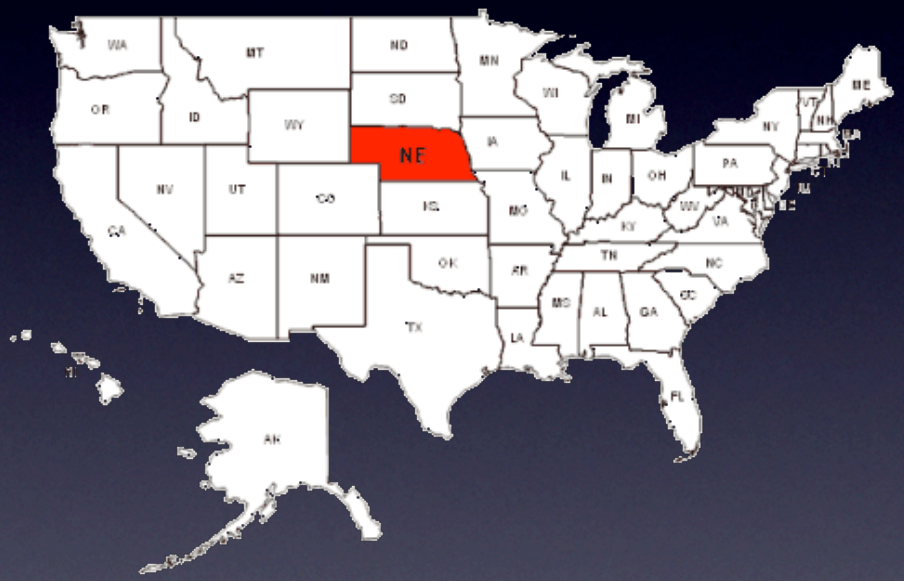
Where is Nebraska?



In the Middle!

My thanks to the American Society Clinical Pathology

Confirms that an individual has demonstrated that s/he possesses the crucial knowledge to perform essential tasks within all areas of the medical laboratory

Worldwide scope

ASCP^{i} Approved Worldwide

In May 2009, the ASCP BOC International Certification Committee (formerly Globalization Committee) approved opening international certification to applicants from around the world. Today, if you meet the requirements for certification, you may sit for the ASCP examination, no matter what your country of education and clinical experience.

International Certification – ASCP^{i}

- **For further information:**
  - Visit the ASCP international website at:
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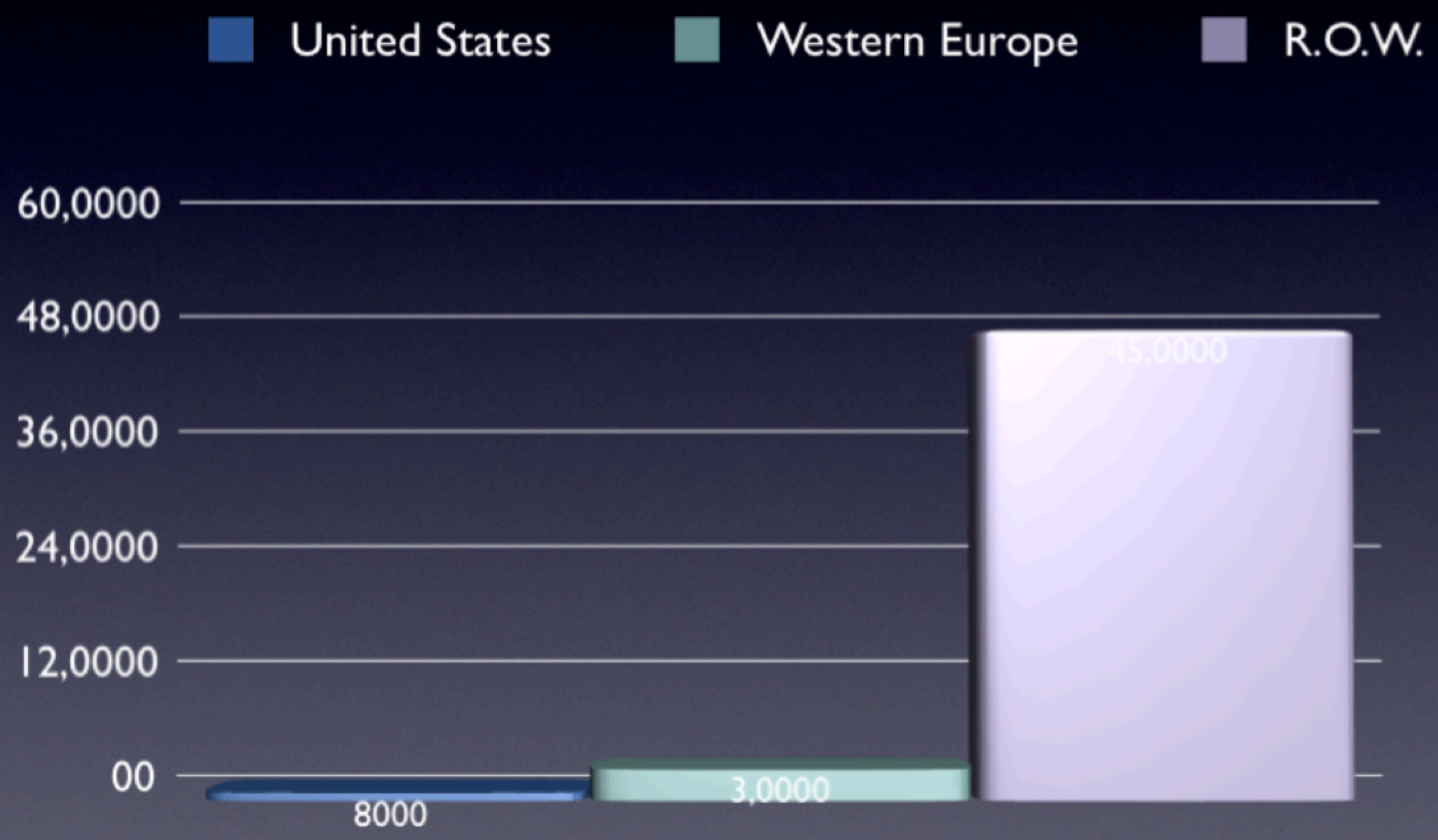
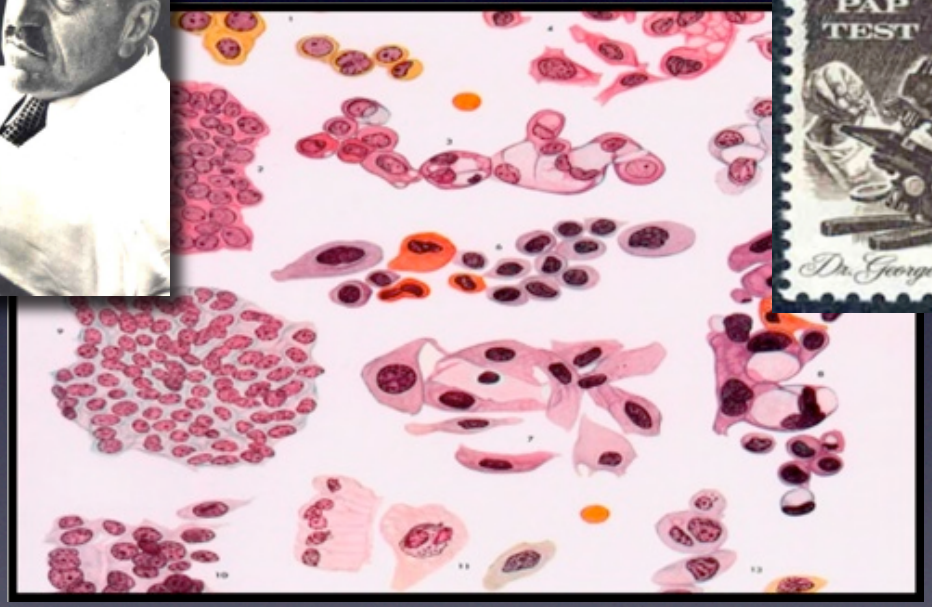
  - **Or contact:** Jennifer Young, CT(ASCP)^{CM}, Senior Manager for ASCP International Certification Activities
  - Jennifer.Young@ascp.org

Cervical Cancer

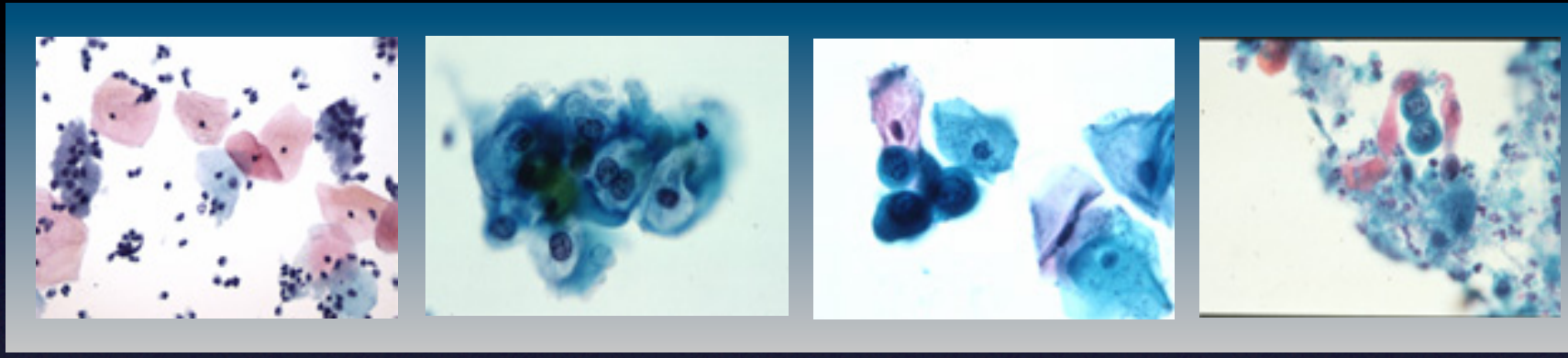


Dr. George N. Papanicolaou pioneered cytology screening



Atlas of Exfoliative Cytology, 1954

Progress to Cervical Cancer

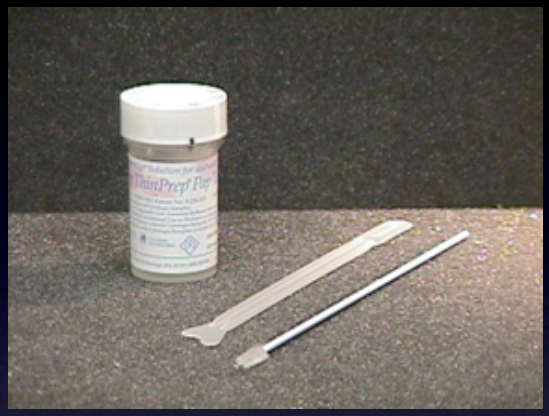


Detection of HSIL is important because it is the immediate precursor of cervical carcinoma

Advancements in Cervical Cancer Prevention

Liquid-based cytology methods were FDA-approved in 1996, 1998 and 2008

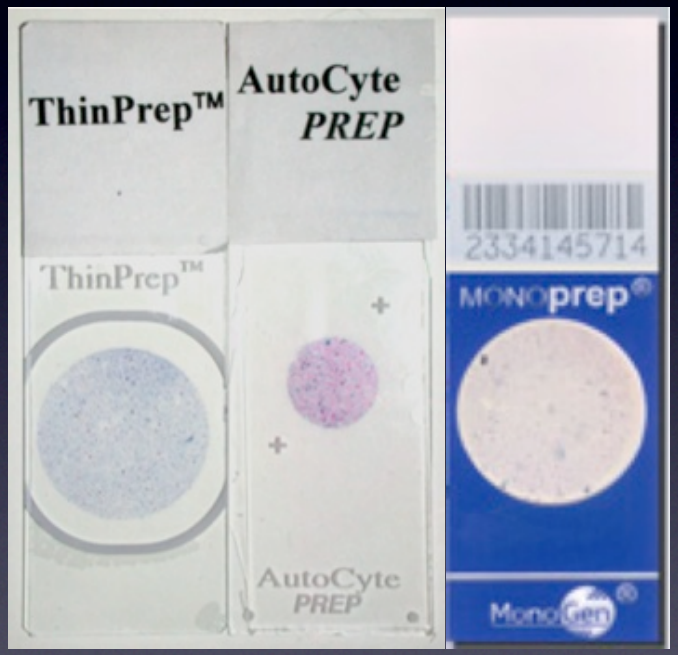
Improved sampling and accuracy of testing



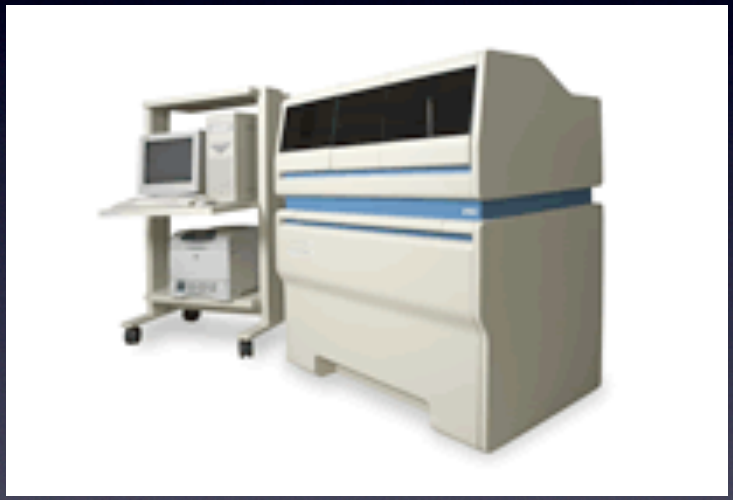
ThinPrep Pap Test

SurePath Pap Test

Liquid method produce a thin-layer of cells on a microscope slide that facilitates computer imaging



FDA approved Cytology imaging systems in 2010



Focal Point System

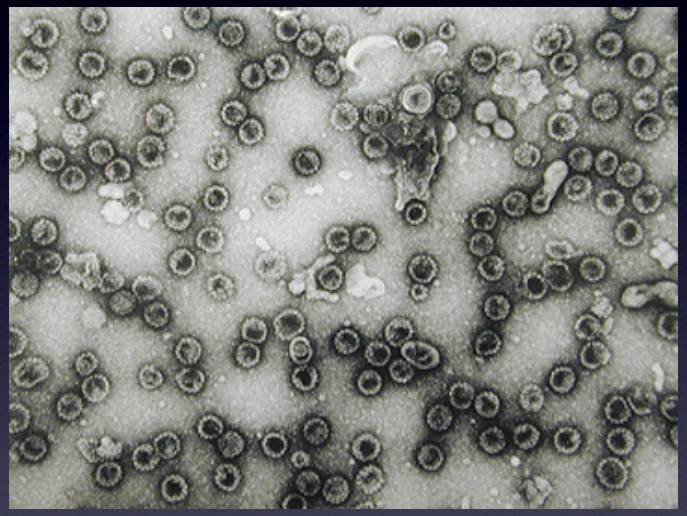
ThinPrep Imaging System

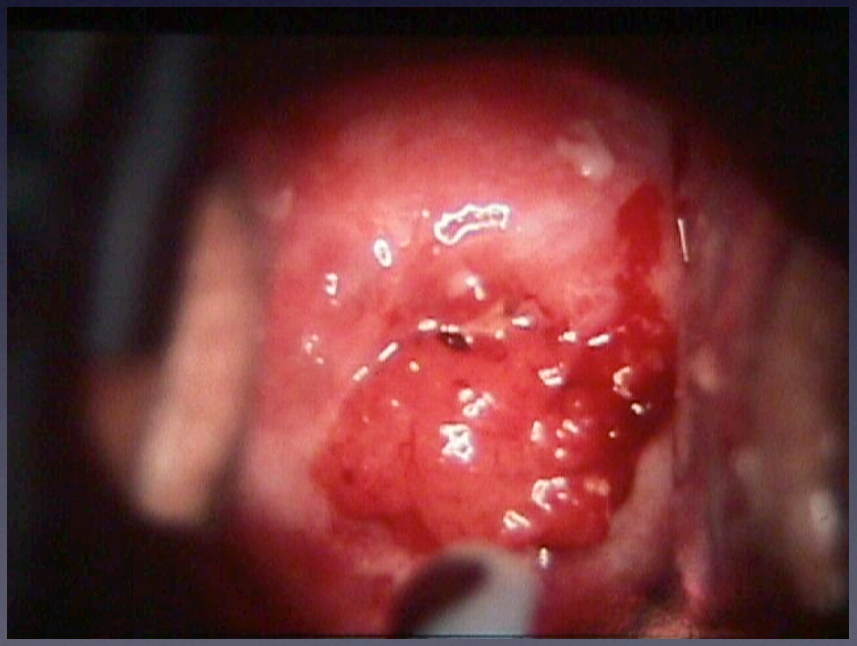
Imaging can also direct the cytologist to potentially abnormal cells

ThinPrep Imaging System

FocalPoint GS System

Human Papilloma Virus (HPV) causes cervical cancer

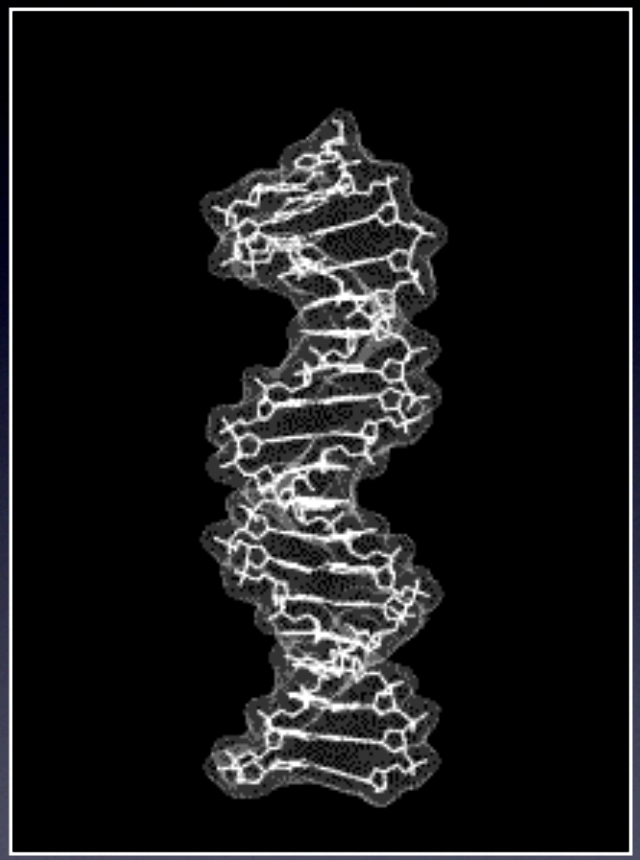




HPV Biology

DNA Virus

7,800 Base-pairs



Early and Late Genes

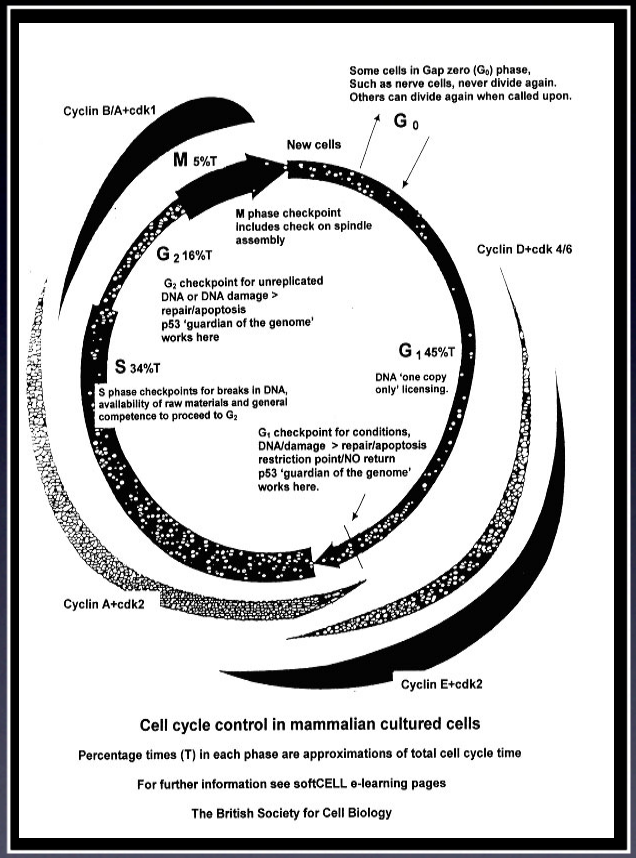
HPV Biology

Three HPV oncogenes play havoc with the cell cycle

E6-p53

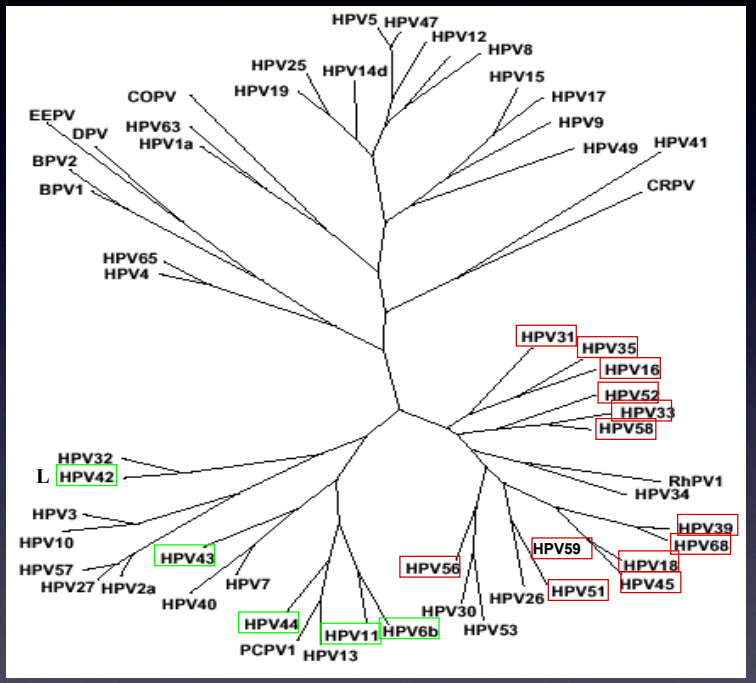
E7-Rb

E2

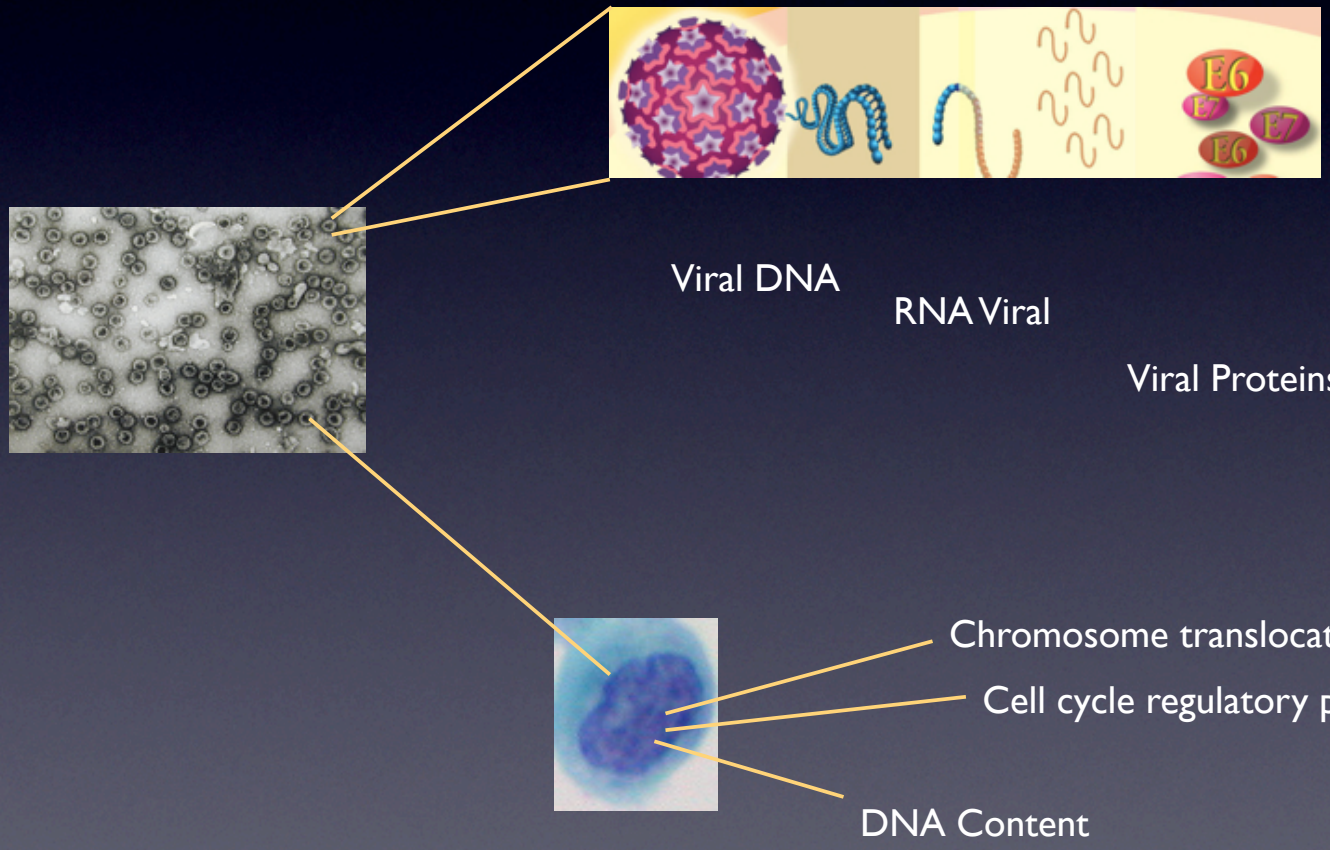


HPV Biology

~130 genotypes



Targets for molecular detection of HPV

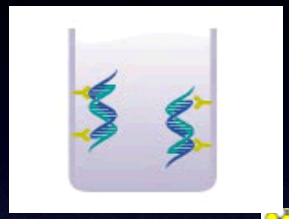


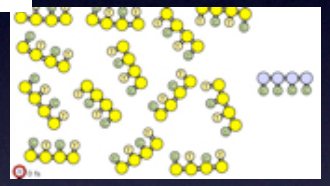
The Cascade: HPV infection to abnormal cytology

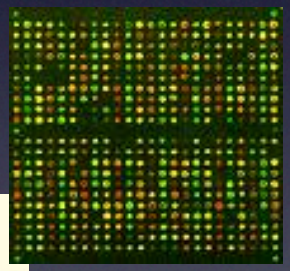
- HPV DNA integrates into the cervical cell genome
- -E6 and E7 mRNA is expressed
- -E6 protein binds to nuclear p53
- -E7 protein binds to nuclear pRB
- -p53 and pRB lose cell cycle regulatory function
- -Nuclear proteins over-expressed (e.g.p16, Ki67)
- -Genomic instability and aneuploidy
- -Nuclei appear abnormal on Pap test

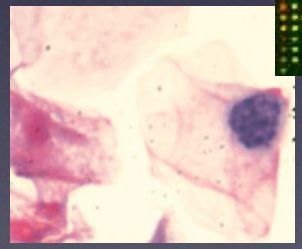
Methods to detect HPV DNA

- Hybrid capture
- Cervista
- PCR Techniques
- Microarray
- Sequencing
- *In-situ* hybridization









Best Sensitivity
Moderate Specificity
Low PPV

FDA Labeling Hybrid Capture 2

When a woman's Pap Test results are mildly abnormal. The HPV-DNA Test is then used to tell whether or not HPV is present at high enough levels to indicate that an HPV infection exists (ASCUS Triage)

When women over age 30 have HPV infections that do not disappear over time (Primary Screening)

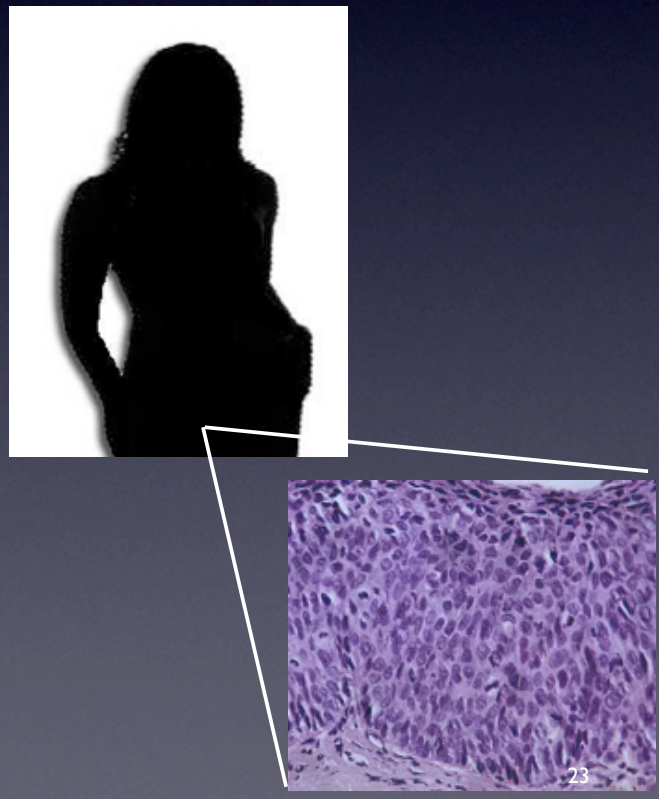
FDA Labeling Cervista

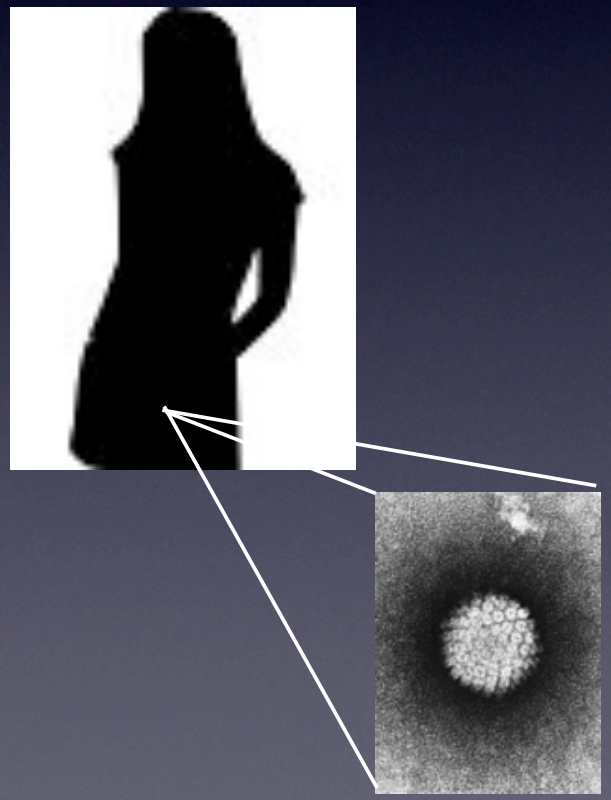
Cervista HPV HR test: To screen patients with atypical squamous cells of undetermined significance (ASCUS) cervical cytology results to determine the need for referral to colposcopy.

Cervista 16/18 HPV test: In women 30 and over, or women of any age with borderline cytology results, to determine the need for additional follow-up and diagnostic procedures.

A “positive” HPV test is a measure of risk, not a diagnosis

A positive HPV DNA test: true lesion, or passive HPV?





HPV DNA typing is potentially more predictive of a true lesion

- **High Risk versus Low Risk**

Typing of High Risk HPV

- 16, 18
- 31, 33, 35, 45, 52, 58
- 39, 51, 56, 59, 68, 73, 82
- 26, 53, 66

Recommendations on HPV DNA test utilization have emerged

- Intended to minimize inappropriate use of HPV testing
- Routine screening > age 30
- ASC-US triage > age 21
- Triage in postmenopausal LSIL
- Postcolposcopy management of certain lesions
- Post treatment surveillance

AJCP

Statement on HPV DNA Test Utilization

Diane Solomon, MD,[1] Jacalyn L. Papillo, CT(ASCP),[2] and Diane D. Davey, MD,[3] on behalf of the Cytopathology Education and Technology Consortium (CETC)

Key Words: Molecular diagnostics; Cytology; Human papillomavirus DNA

Testing for carcinogenic or high-risk human papillomavirus (HPV) DNA has proven utility in cervical cancer screening and in many aspects of clinical management for cervical cancer prevention. However, inappropriate testing increases costs without benefit and potentially results in overtreatment of women. This statement was developed by the Cytopathology Education and Technology Consortium and has been endorsed by additional professional medical societies (listed at the end of the statement). It is intended as a concise, convenient summary of clinical indications for HPV DNA test utilization based on the American Cancer Society 2002 screening recommendations[1] and interim guidance[2] and the 2006 American Society for Colposcopy and Cervical Pathology (ASCCP) consensus management guidelines.[3] Circumstances in which HPV DNA testing is considered appropriate and when such testing is generally not appropriate are outlined. This statement and Table 1 are intended to serve as an educational tool and reference to improve management of women and reduce inappropriate use of HPV tests.

1. High-risk (oncogenic) HPV DNA testing is appropriate in the following circumstances:
   1.1. Routine cervical cancer screening in conjunction with cervical cytology (dual testing or cotesting) for women 30 years or older:
      1.1.1. For women who are cytology-negative but HPV-positive, repeat both tests in 12 months. (As of March 2009, the US Food and Drug Administration approved an HPV-16/18 genotyping test; per ASCCP guidelines,[3] HPV-16 positive and/or HPV-18 positive women 30 years or older are referred directly to colposcopy.)
      1.1.2. For women who are both cytology- and HPV-negative, repeat both tests only after a 3-year interval.

Table 1
Appropriate Uses of HPV Testing in Screening and Triage*

Age (y)	Routine Screening	Initial Triage: ASC-US	LSIL	ASC-H	AGC†	HSIL
≤20	2.1	2.3	2.3	2.3	2.3	2.3
21-29	2.1	**1.2**	2.4	2.5	2.5	2.5
30+	**1.1**‡	**1.2**	2.4	2.5	2.5	2.5
Postmenopausal	**1.1**‡	**1.2**	**1.3**	2.5	2.5	2.5

AGC, atypical glandular cells; ASC-H, atypical squamous cells, cannot exclude HSIL; ASC-US, atypical squamous cells of undetermined significance; HSIL, high-grade squamous intraepithelial lesion; HPV, human papillomavirus; LSIL, low-grade squamous intraepithelial lesion.
* Boldface type indicates HPV testing is appropriate. The numbers in the table cells refer to the text outline.
† Note that for AGC results, HPV testing is not to be used for triage to decide whether to refer to colposcopy; however, HPV testing may be done at the time of colposcopy to guide postcolposcopy management.
‡ For women 30 years or older who are cytology- and HPV-negative, repeat both tests only after a 3-year interval.

Am J Clin Path May, 2009

Measuring E6 E7 HPV mRNA

- Pre TECT Norchip
- BioMerieux
- APTIMA Genprobe

Moderate Sensitivity
High Specificity
Good PPV

Proteins are HPV biomarkers

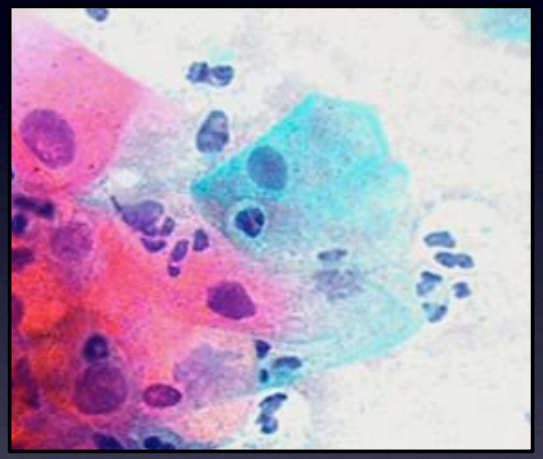
- HPV DNA integrates into the cervical cell genome
- -E6 and E7 mRNA is expressed
- -E6 protein binds to nuclear p53
- -E7 protein binds to nuclear pRB
- -p53 and pRB lose cell cycle regulatory function
- -HPV and host protein expressed (L1, p16, Ki67)
- -Genomic instability (mcm, topoisomerase)
- -Aneuploidy nuclear abnormality on Pap test

Potential applications of biomarker proteins

- ASC-US triage
- Locating abnormal cells of interest
- Predicting behavior of lesions

Case 1

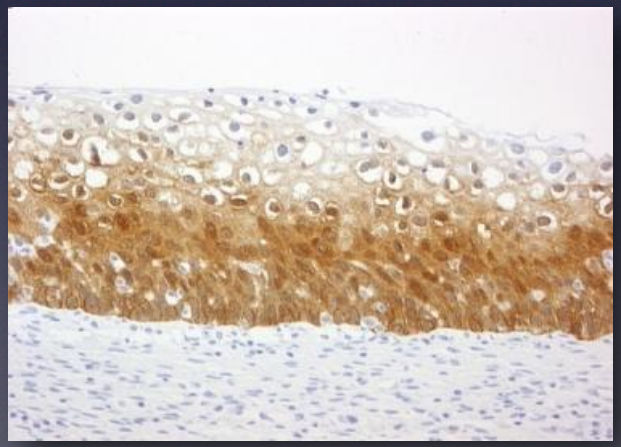
- Women having Atypical Squamous cells of Undetermined Significance (ASC-US)



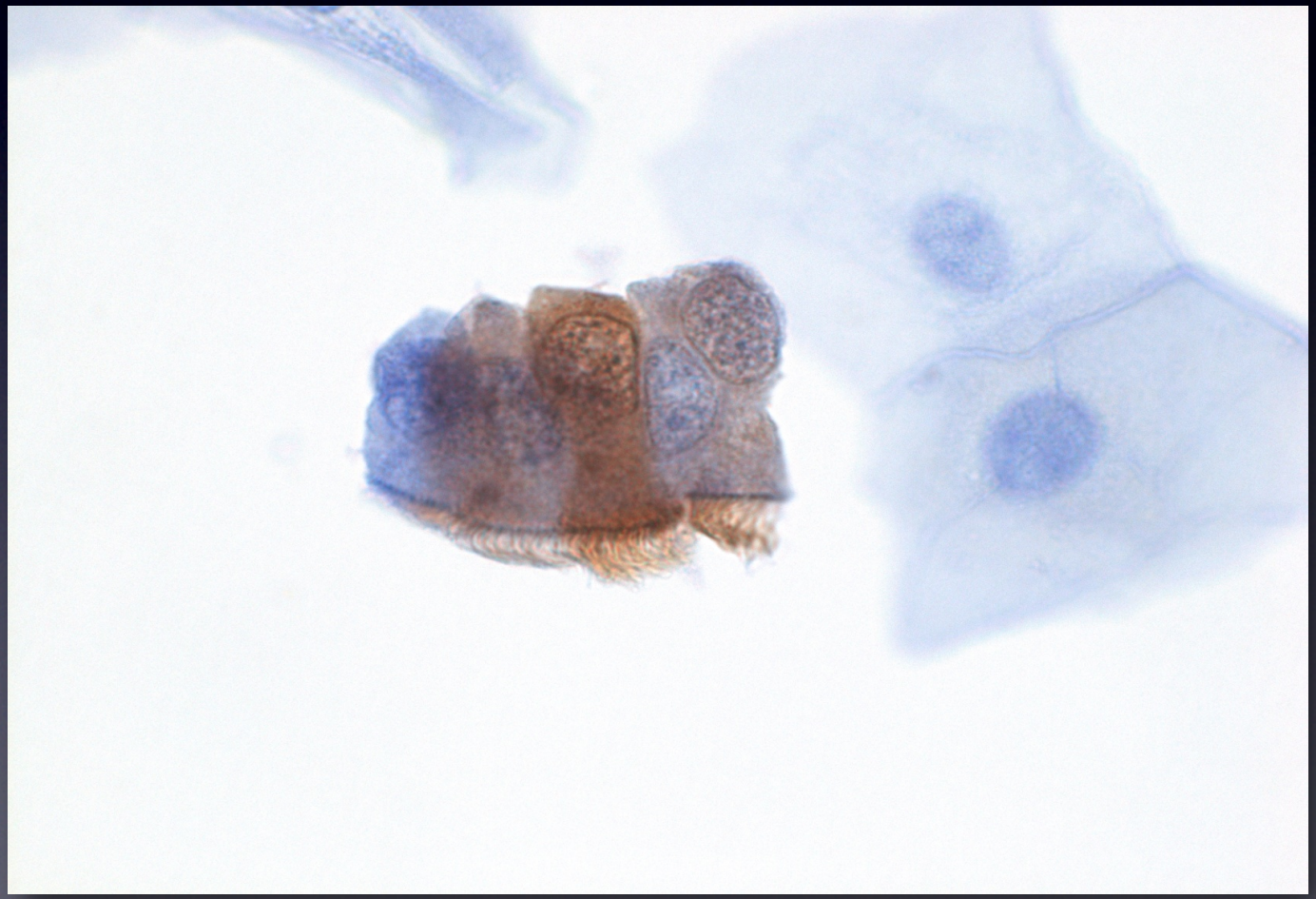
- Use of p16 as tool to determine management

p16

- Cellular protein, involved in cell-cycle control
- Anti-proliferative effect in physiologically normal cells
- Over-expression directly linked to oncogenic transformation of cervical cells induced by persistent HR-HPV infections
  - Independent of HR-HPV type
  - Independent of patient age

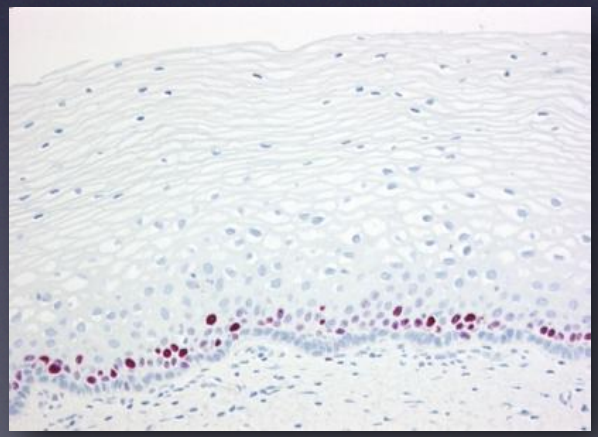


P16 can be expressed in normal endocervical cells



Ki67 marks nuclei in the proliferative phase

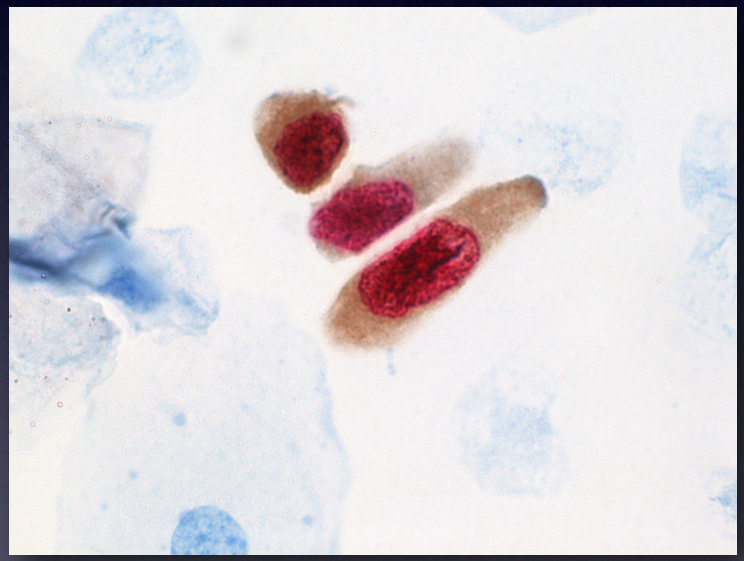
- Nuclear protein that can be detected in proliferating cells
- Expression restricted to the G1-, S-, G2 and M-phase of the cell cycle
  - Marker of cell proliferation
- No expression in quiescent cells
  - Absent in G0-phase of the cell cycle



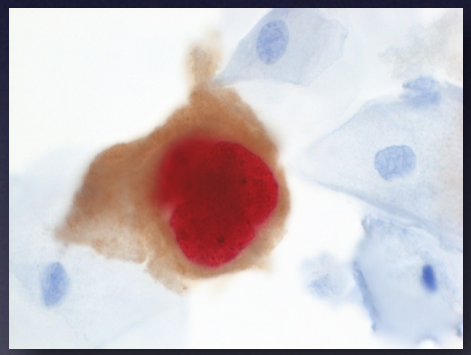
Proliferating cells in normal squamous cervical epithelium show nuclear Ki-67 staining

CINtec® *PLUS*; p16 *PLUS* Ki-67

- Co-detection of p16 PLUS Ki-67 within the same cell
  - Serves as an indicator of cell cycle de-regulation, which occurs during HR-HPV induced oncogenic transformation
    - Brown cytoplasmic staining, indicating p16 over-expression
    - Red nuclear staining, indicating expression of Ki-67



Interpretation of CINtec® *PLUS*



Screening for p16/Ki-67
Dual-stained cells

One or more Dual-stained
Cell(s) present?

YES

CINtec® PLUS
Positive

NO

CINtec® PLUS
Negative

AJCP July 2010

The Sensitivity and Specificity of p16^{INK4a} Cytology vs HPV Testing for Detecting High-Grade Cervical Disease in the Triage of ASC-US and LSIL Pap Cytology Results

Karin J. Denton, MD,[1] Christine Bergeron, MD, PhD,[2*] Petra Klement,[3] Marcus J. Trunk, MD,[3†] Thomas Keller, PhD,[4] and Ruediger Ridder, PhD,[3] for the European CINtec Cytology Study Group[‡]*

Key Words: Cervical intraepithelial neoplasia; Cervical cytology; Atypical squamous cells of undetermined significance; ASC-US; Low-grade squamous intraepithelial lesion; LSIL; Triage; p16^{INK4a}; Immunocytochemistry; Human papillomavirus; HPV

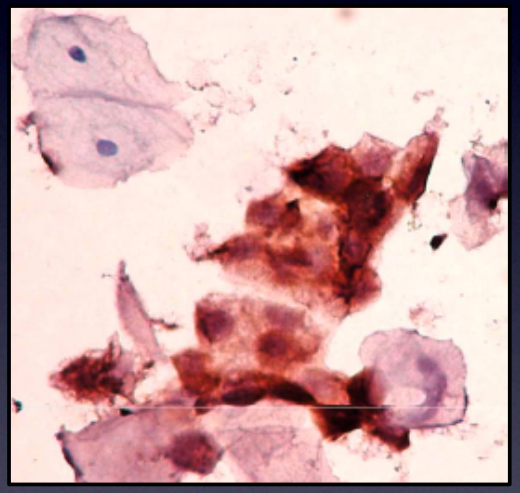
DOI: 10.1309/AJCP3CD9YKYFJDQL

Editorial Mark Stoler, MD

"Despite these limitations and biases, the punch line is clear: p16 immunocytochemical analysis as described in this study is just as sensitive as high-risk HPV testing for the identification of biopsy-proven high-grade CIN with approximately twice the specificity. This is a remarkable result because it implies that potentially, if demonstrated by a proper prospectively designed clinical trial that addresses some of the limitations, p16 triage of ASC-US and, may be even more importantly, LSIL cytologic results would find just as many CIN 3 cases as the current clinical practice under American Society for Colposcopy and Cervical Pathology guidelines while referring approximately 25% to 50% fewer patients to colposcopy."

Anti-E6, -E7 Oncoprotein can highlight abnormal cells

Cervical cells infected by HPV will express E6 and E7 proteins. Immunocytochemistry for these proteins may be used to identify abnormal cells

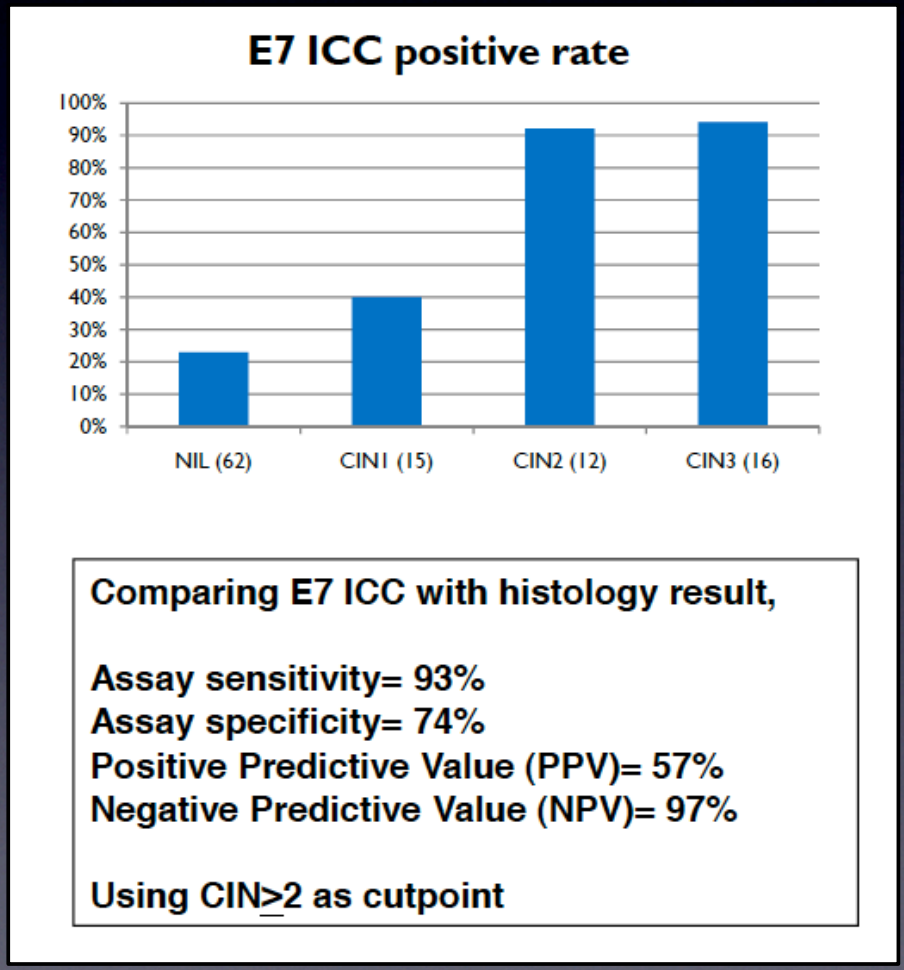


HSIL Anti-E7

Courtesy Dr. Shuling Cheng
OncoHealth Corporatoion

OncoHealth E7 Study

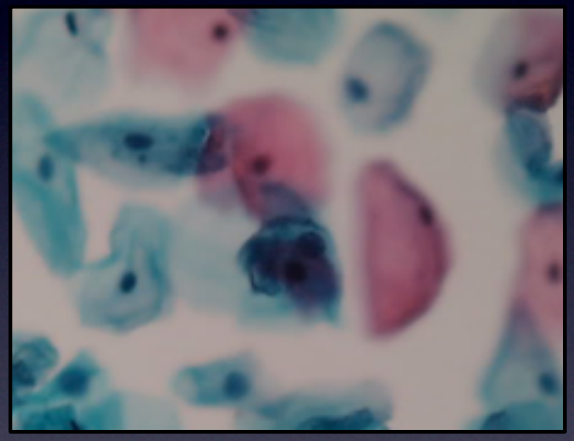
International Papilloma Virus Conference 2009



Courtesy Dr. Shuling Cheng
OncoHealth Corporatoion

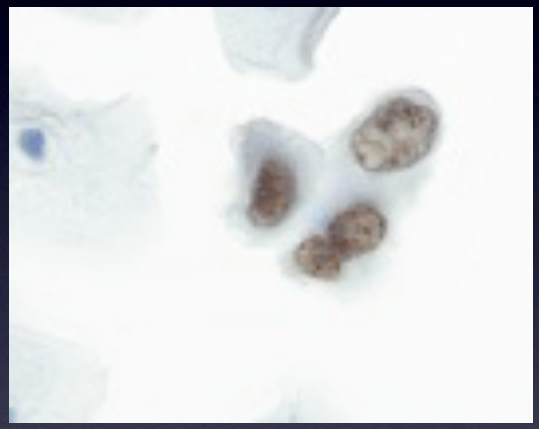
Case 2

- Women thought to be at high-risk of having abnormality because of Pap history.
- Use of ProExC as tool to identify abnormal cells



ProExC

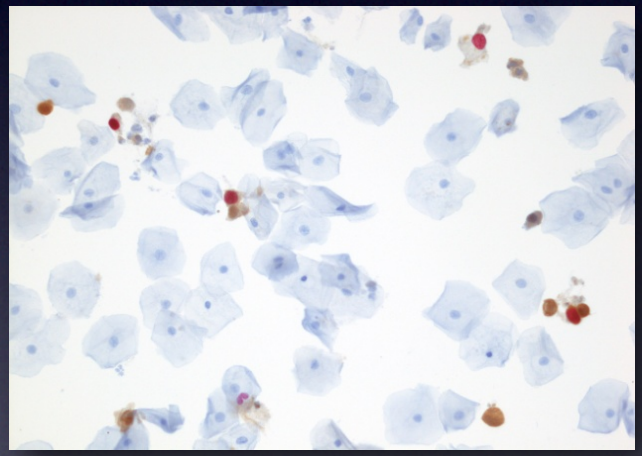
- Anti-mcm2
- Anti-Top2A
- Expression indicates “aberrant S-phase induction”



Moderate Sensitivity
Moderate Specificity
High PPV

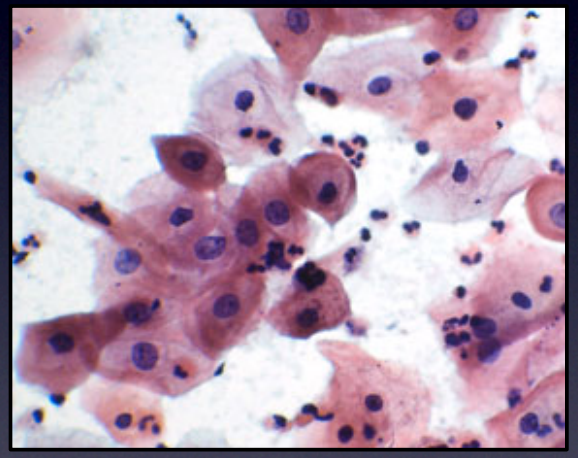
Biomarkers can be used as an alternative to Pap stain

- Abnormal cells may be located (with or without imaging) based on protein expression:
- - ProExC
- - P16
- - E6 or E7 protein



Case 3

- Woman with LSIL. How should they be managed?



- L1 protein expression to guide treatment

There is interest in markers for prognostic use

Prognostic significance of the detection of human papilloma virus L1 protein in smears of mild to moderate cervical intraepithelial lesions

D. Rauber [a], G. Mehlhorn [a], P.A. Fasching [a], M.W. Beckmann [a], S. Ackermann [a,b,*]

[a] *Department of Obstetrics and Gynecology, Erlangen University Hospital, Erlangen, Germany*
[b] *Department of Obstetrics and Gynecology, Darmstadt General Hospital, Darmstadt, Germany*

Received 12 April 2006; received in revised form 19 April 2008; accepted 11 May 2008

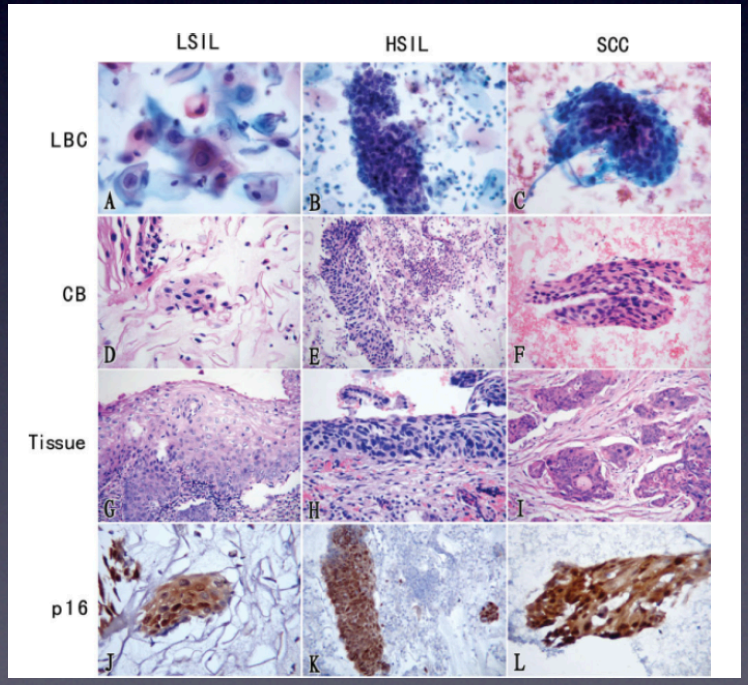
- Will an ASCUS case progress to a higher grade lesion or regress? Will LSIL progress to HSIL? Can a LSIL lesion be managed conservatively?

Examples of L1 immunohistochemistry

Original Article

Diagnostic Value of p16INK4A, Ki-67, and Human Papillomavirus L1 Capsid Protein Immunochemical Staining on Cell Blocks From Residual Liquid-Based Gynecologic Cytology Specimens

Li Yu, MD[1,2]; Liantang Wang, MD, PhD[1]; Juemin Zhong, MD[1]; and Shangwu Chen, MD, PhD[3]

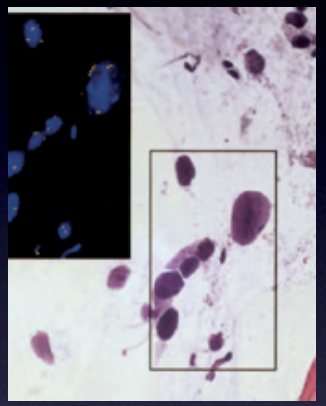


Methods that might be predictive

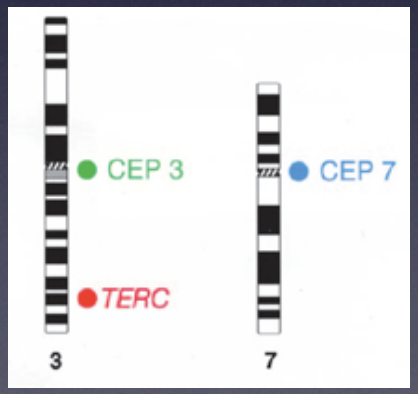
- Persistent presence of high-risk HPV
- Integration of HPV
- Specific chromosomal abnormalities (3q26)
- L1 expression
- P53 related proteins: P63, TP73
- P16 over-expression

Translocation- 3q26

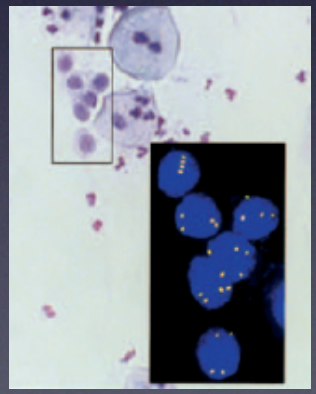
- Location of the telomerase gene TERC
- Amplification of TERC associated with HSIL



LSIL with progression risk



False-negative Pap



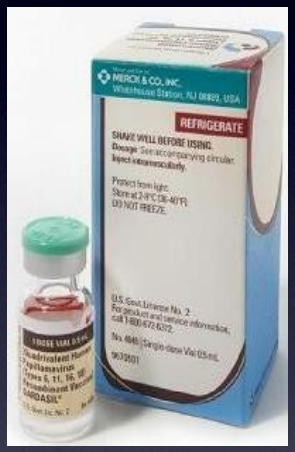
Hesselmeryer-Haddad et al 2005

Limitations of predictive markers

- None are absolute e.g. the marker is often express of 5% or more of normal cells
- Liability concerns related to not treating some lesions

HPV Vaccination may also affect cervical screening by altering disease prevalence

- VLP Produced in yeast or baculovirus system
- Highly immunogenic (40x) for L1 protein of HPV



Thank you

A copy of this presentation can be downloaded at:

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