The Impact of Primary HPV Screening on the Cytology Laboratory

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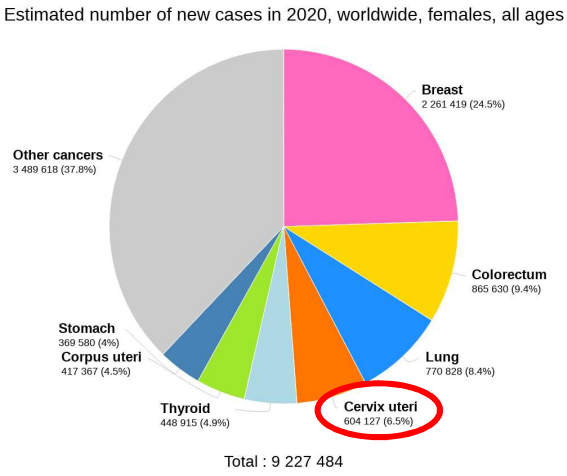
Brigham and Women's Hospital
Harvard Medical School

Outline

- Cervical Cancer Screening
- Human Papilloma Virus (HPV)
- HPV Testing Platforms
- Primary HPV Screening & Laboratory Workflows

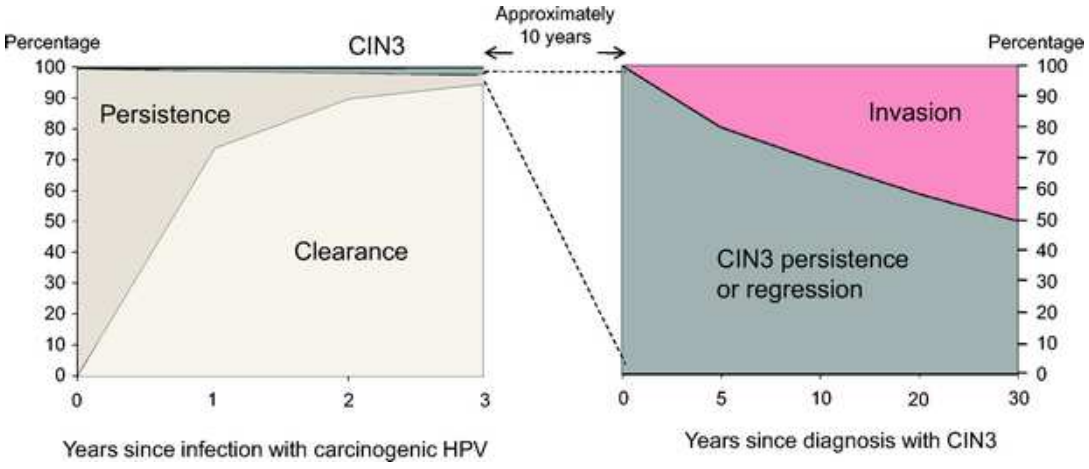
Cervical Cancer Incidence

- Common worldwide:
  - >600,000 cases
  - >340,000 deaths
- United States:
  - ~14,500 cases
  - ~4,300 deaths



http://globocan.iarc.fr

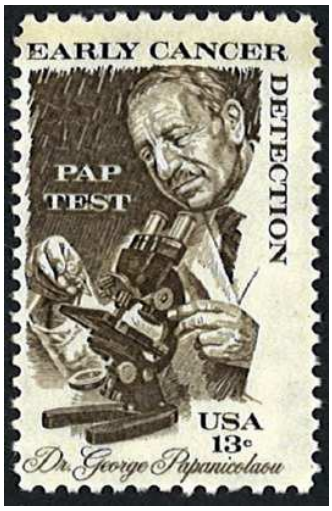
Development of Invasive Carcinoma

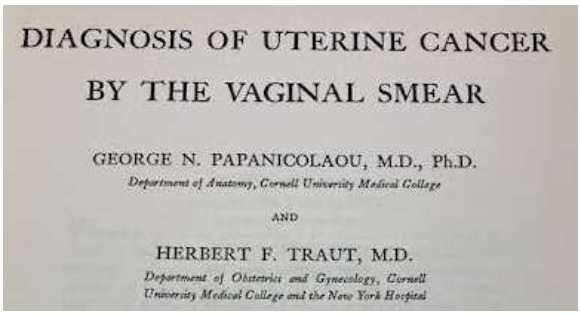


Schiffman M & Wentzensen N. Ob & Gyn (2010) **116:** 177

The Pap Test

- **Early 1900s:** Cervical Cancer was the No. 1 cancer related cause of death for women (~36.3 deaths/100k)
- Developed by Dr. George Papanicolaou
- 1920s began studying vaginal smears from guinea pigs
- Identified cancerous cells in the late 1920s
- Finding largely overlooked for the next 10+ years



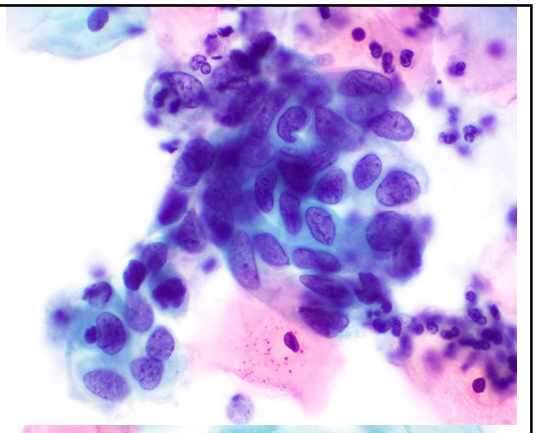

DIAGNOSIS OF UTERINE CANCER
BY THE VAGINAL SMEAR

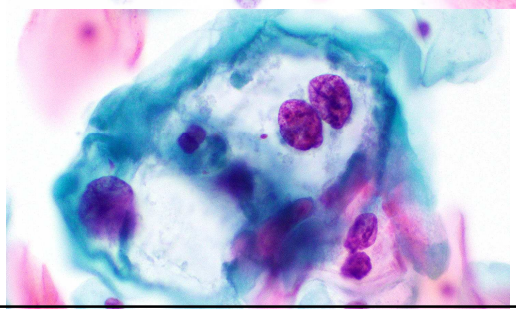
GEORGE N. PAPANICOLAOU, M.D., Ph.D.
Department of Anatomy, Cornell University Medical College

AND

HERBERT F. TRAUT, M.D.
Department of Obstetrics and Gynecology, Cornell University Medical College and the New York Hospital

- Descriptive atlas of cells in vaginal smears
- Examined 7,000-10,000 smears from ~3000 adult women
- 193 carcinomas by histology
  - All but 11 cases had “malignant” cells identified by vaginal smear





Early 1900s: Cervical Cancer is the No. 1 Cancer related cause of death for women (~36.3 deaths/100k)

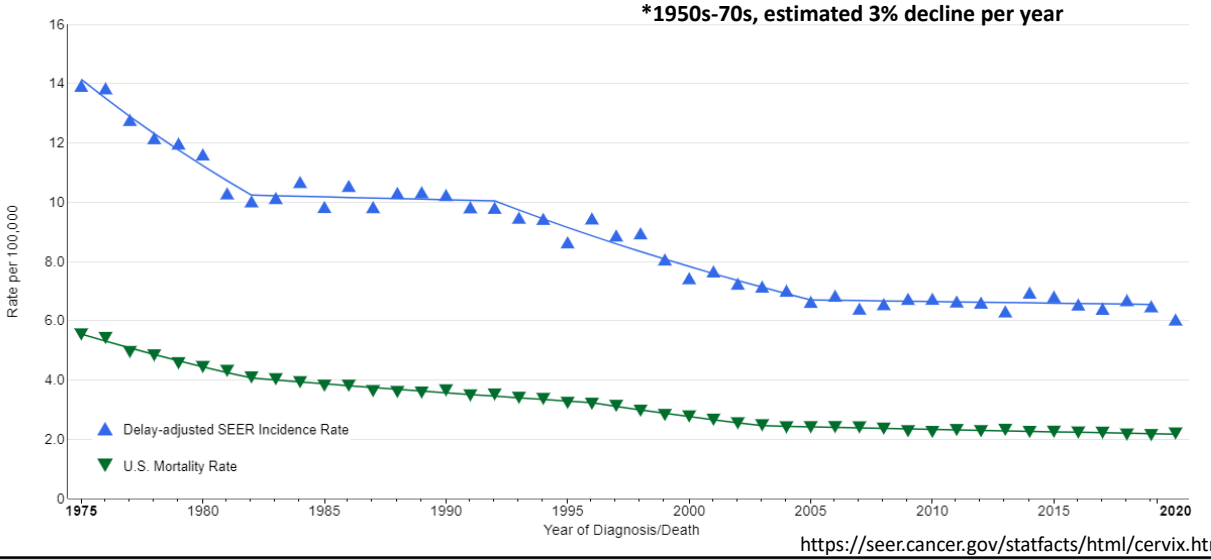
1927-29: Dr. Papanicolaou identifies malignant cervical cancer cells by vaginal smear

1943: Drs. Papanicolaou and Traut publish Diagnosis of Uterine Cancer by vaginal smear.

1950s: Annual screening with Pap smears begins

Longterm Trends in Cervical Cancer Incidence

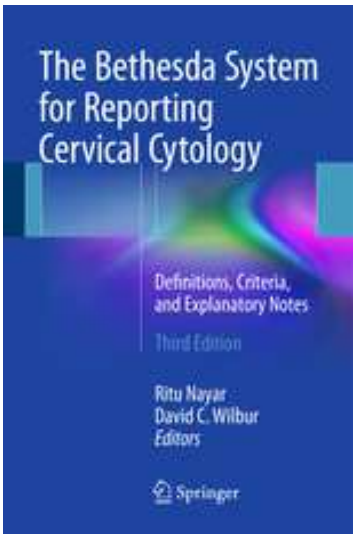
*** ~36 per 100,000 women in 1930s**
***1950s-70s, estimated 3% decline per year**



https://seer.cancer.gov/statfacts/html/cervix.html

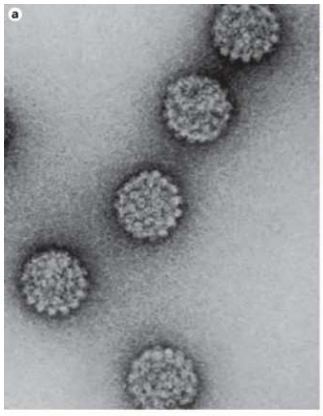
The Bethesda System

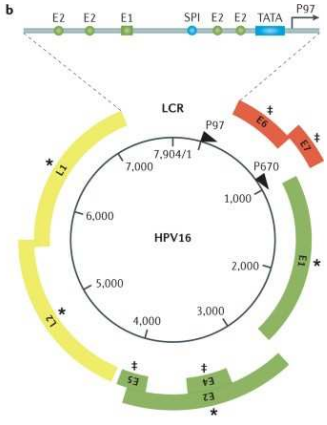
- Introduction of standardized reporting:
  - 1st edition: 1994
  - 2nd edition: 2004
  - 3rd edition: 2014
- Majority of abnormal results were ASCUS or LSIL
- At the time of introduction of the Bethesda system there was no clear way to triage these patients
  - Colposcopy
  - Repeat pap test



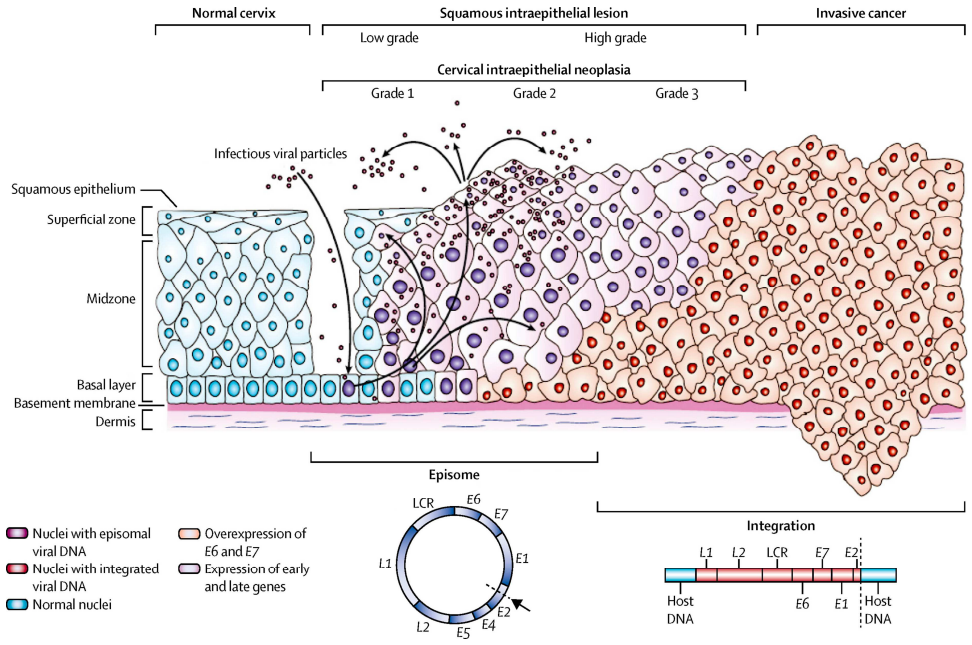
Human Papilloma Virus

- Linked to cervical cancer in 1974
- Most common sexually transmitted infection in the United States
- Non-enveloped double stranded DNA virus with >100 known types
- ~14 high-risk HPV types:
  - **16**, **18**, **31**, **33**, 35, 39, **45**, 51, **52**, 56, **58**, 59, 66, and 68
- Types 16 and 18 responsible for ~75% of cervical cancer worldwide





Schiffman M, et al. Nat. Rev. Dis. (2016) 2:1



Cohen PA, et al. The Lancet (2019) **393**: 169

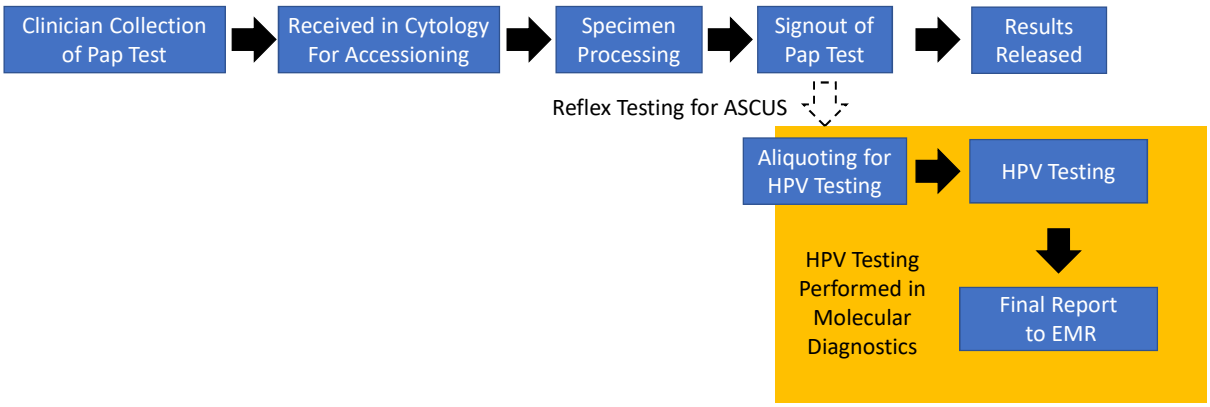
ASCUS/LSIL Triage Study (ALTS) for Cervical Cancer

- NCI sponsored randomized multicenter trial for the management of women with ASCUS or LSIL (1996-2000)
- ~5000 women with ASCUS or LSIL randomized to: immediate colposcopy, repeat cytology, or HPV testing for detection of CIN3+ disease
  - HPV testing is sensitive for detecting CIN2/3 lesions in women with an ASCUS pap test
  - HPV testing is not useful for triage of women with LSIL pap

Solomon D, et al. JNCI (2001) **94**: 293-299
ALTS Group. JNCI (2000) **92**: 397-402

Historic Gyn Cytology Workflow

Reflex HPV Testing Gyn Workflow



Early 1900s: Cervical Cancer is the No. 1 Cancer related cause of death for women (~36.3 deaths/100k)

1927-29: Dr. Papanicolaou identifies malignant cervical cancer cells by vaginal smear

1943: Drs. Papanicolaou and Traut publish Diagnosis of Uterine Cancer by vaginal smear.

1950s: Annual screening with Pap smears begins

1974: HPV hypothesized to be involved in cervical cancer carcinogenesis

1983: HPV types 16/18 linked to cervical cancer

1988: Introduction of the Bethesda System

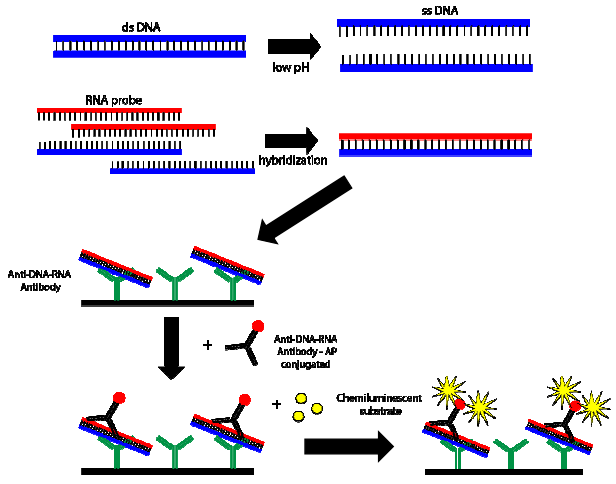
1999: Hybrid Capture 2 receives FDA approval for ASCUS triage

2001: HPV testing recommended for ASCUS triage by ASCCP.

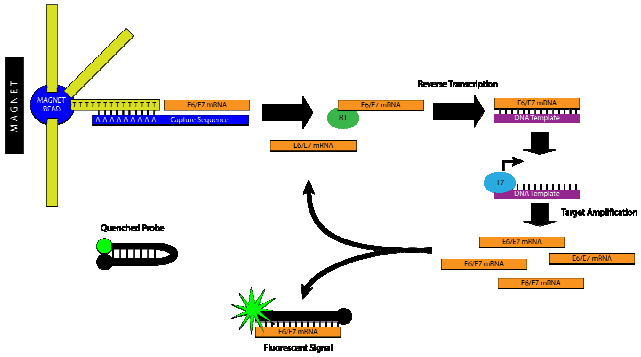
2001-2018: FDA approval of additional HPV assays

Clinical HPV Assays

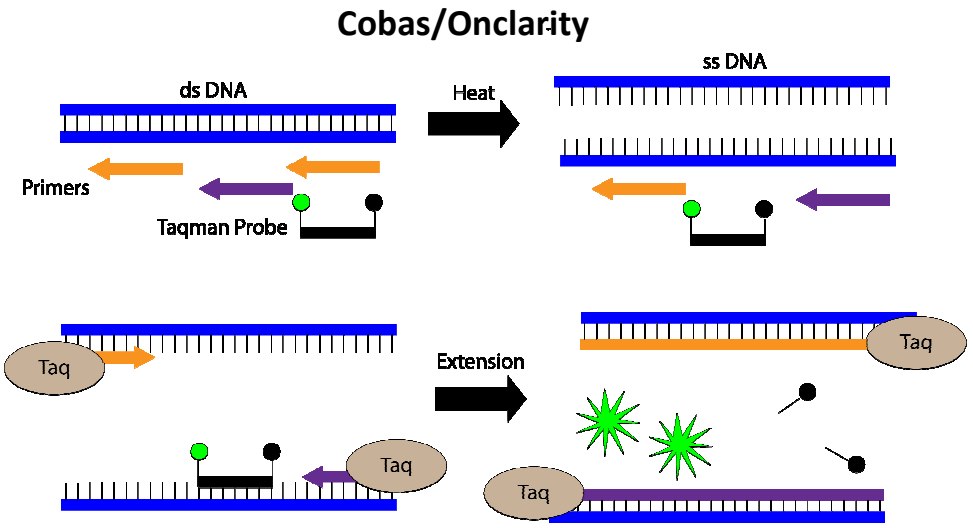
Hybrid Capture 2



Aptima



Clinical HPV Assays



Test	Hybrid Capture II	Aptima	Cobas	BD Onclarity
Manufacturer	Qiagen	Hologic	Roche	Becton Dickinson
FDA approved for reflex/co-testing	2001	2011	2011	2018
Method	DNA (non-PCR) Signal amplification: full genome probe	**mRNA** *in vitro* transcription: E6/E7 gene target	**DNA** (qPCR based): L1 gene target	**DNA** (qPCR based): E6/E7 gene target
Genotypes detected	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68	16*, 18*, 31, 33, 35, 39, 45*, 51, 52, 56, 58, 59, 66, and 68	16*, 18*, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68	16*, 18*, 31, 33, 35, 39, 45*, 51, 52, 56, 58, 59, 66, 68
Clinical trial	ASC-US/LSIL Triage Study (ALTS), 2006 CAP	CLEAR trial	ATHENA	Onclarity trial
Sensitivity for CIN2/3	63.6%-100%	55.3%-100%	71.1%-99%	85.7%-100%
Specificity for CIN2/3	6.2%-98.4%	28.8%-99.2%	24%-86.2%	17%-98.8%
Built-in internal control	No	Yes, HPV16 E6/E7 transcript is spiked into each reaction at the target capture step	Yes (ß-globin)	Yes (ß-globin)

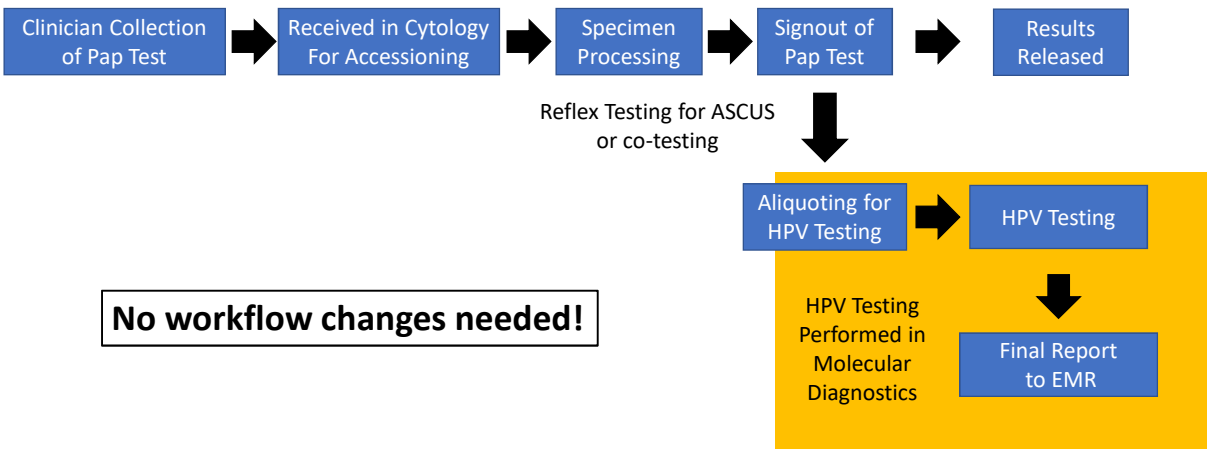
Modified from Salazar KL, et al. JASC (2019) **8**: 284

Co-Testing

- Pap test with HPV testing
- Increased overall sensitivity of the pap test
- Initially approved for HC2 by FDA in 2003
- Recommended in the US for women aged 30-65 by multiple organizations in 2012 and 2013
- Widely adopted with changing recommendations:
  - Academic center (JHU): 78% in 2013
  - State-wide (NM): 84.3% in 2019

Cuzick J, et al. Gyn Onc (2021) **162**: 555
Silver MI, et al. Can. Causes Con. (2018) **29**: 43

Gyn Cytology Workflow



No workflow changes needed!

Clinical Review & Education

JAMA | US Preventive Services Task Force | RECOMMENDATION STATEMENT

Screening for Cervical Cancer

US Preventive Services Task Force Recommendation Statement

US Preventive Services Task Force

"…USPSTF now recommends screening every 5 years with hrHPV testing alone as an alternative to screening every 3 years with cytology alone…. These are the 2 preferred screening strategies…. Cotesting as an alternative strategy has demonstrated similar effectiveness, although it may result in more tests and procedures compared with either cytology or hrHPV testing alone."

USPSTF. JAMA (2018) **320**: 674

Rationale for Primary HPV Testing

- A negative HR-HPV test result has a lower cumulative incidence of CIN3+ than cytology at 3- or 5-year follow-up
- Reduce harm by decreasing the number of follow-up tests/colposcopies
- Primary HPV screening with triage using genotyping AND cytology increases detection of CIN3+ over cytology alone
- Cytology fails to detect a significant portion of CIN3+ lesions in younger patients
- HR-HPV testing alone is predicted to prevent 1 additional cancer case per 1000 screened individuals compared to cytology alone
- Reduced number of lifetime screenings
- Reduced healthcare costs

USPSTF. JAMA (2018) **320**: 674

Cervical Cancer Screening for Individuals at Average Risk: 2020 Guideline Update from the American Cancer Society

Elizabeth T. H. Fontham, MPH, DrPH[1]; Andrew M. D. Wolf, MD[2]; Timothy R. Church, PhD[3]; Ruth Etzioni, PhD[4,5]; Christopher R. Flowers, MD, MS[6]; Abbe Herzig, PhD[7]; Carmen E. Guerra, MD[8]; Kevin C. Oeffinger, MD[9]; Ya-Chen Tina Shih, PhD[10]; Louise C. Walter, MD[11,12]; Jane J. Kim, PhD[13]; Kimberly S. Andrews, BA[14]; Carol E. DeSantis, MPH[15]; Stacey A. Fedewa, PhD, MPH[15]; Deana Manassaram-Baptiste, PhD, MPH[14]; Debbie Saslow, PhD[14]; Richard C. Wender, MD[16]; Robert A. Smith, PhD[14]

"The ACS now recommends primary HPV testing at a 5-year interval as the preferred screening strategy *for all individuals being screened*."

Fontham ETH, et. al. CA Cancer J Clin. (2020) **70:** 321

Age	2020 ACS	2018 USPSTF (ACOG/ASCCP/SGO)
<21	Not recommended	Not recommended
21-29	Starting at age 25: * Primary HPV testing alone, every 5 years **(preferred)** **or** * Co-testing, every 5 years **or** * Pap test only, every 3 years	Starting at age 21: * Pap test only every 3 years
30-65	* Primary HPV testing alone, every 5 years **(preferred)** **or** * Co-testing, every 5 years **or** * Pap test only, every 3 years	* Primary HPV testing alone, every 5 years **or** * Pap test only, every 3 years **or** * Co-testing, every 5 years
>65	Not recommended*	Not recommended#

Fontham ETH, et al. CA Cancer J Clin (2020) **70**:321-346
USPSTF. JAMA (2018) **320**:674-686

Test	Hybrid Capture II	Aptima	Cobas	BD Onclarity
Manufacturer	Qiagen	Hologic	Roche	Becton Dickinson
FDA approved for reflex/co-testing	2001	2011	2011	2018
FDA approved for primary screening	N/A	N/A	**2014 (ThinPrep)** **2018 (Surepath)**	**2018 (SurePath)** **2023 (ThinPrep)**
Method	DNA (non-PCR) Signal amplification: full genome probe	**mRNA** *in vitro* transcription: E6/E7 gene target	**DNA** (qPCR based): L1 gene target	**DNA** (qPCR based): E6/E7 gene target
Genotypes detected	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68	16*, 18*, 31, 33, 35, 39, 45*, 51, 52, 56, 58, 59, 66, and 68	16*, 18*, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68	16*, 18*, 31, 33, 35, 39, 45*, 51, 52, 56, 58, 59, 66, 68
Clinical trial	ASC-US/LSIL Triage Study (ALTS), 2006 CAP	CLEAR trial	ATHENA	Onclarity trial
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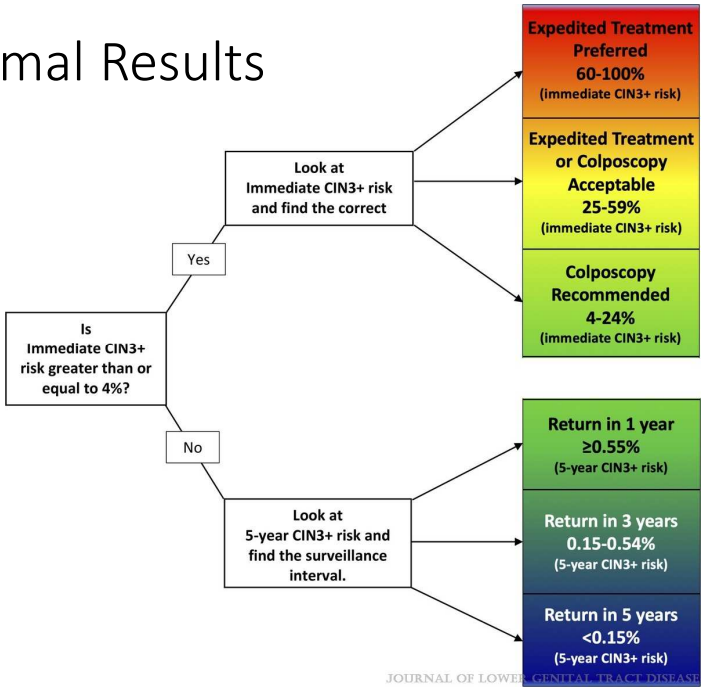
Modified from Salazar KL, et al. JASC (2019) **8**: 284

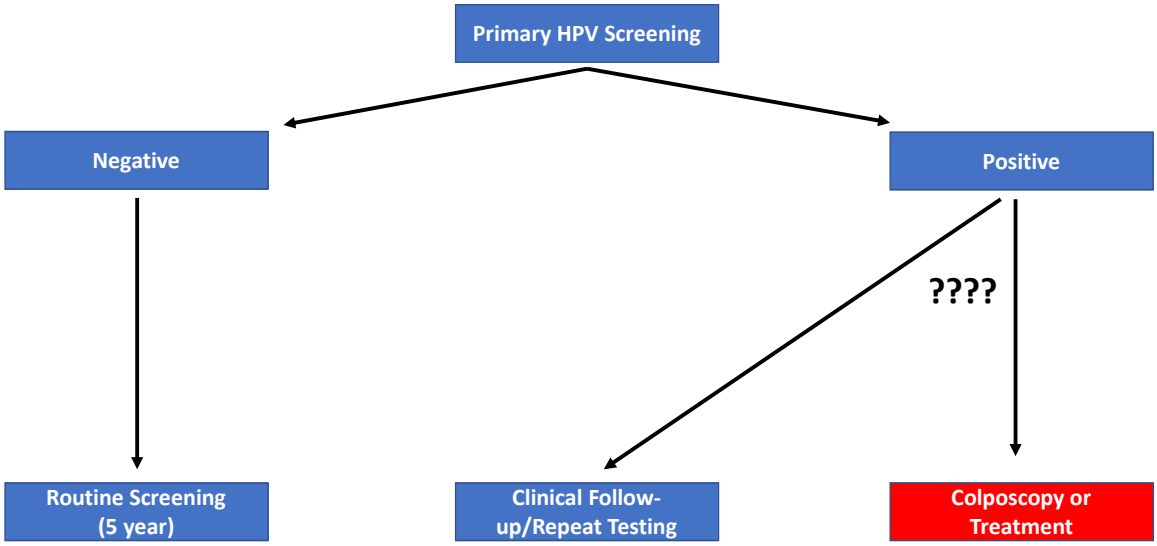
Management of Abnormal Results

- Updated 2019 ASCCP Guidelines
- Paradigm shift → Risk based management
- Triage point is *immediate* CIN3+ risk of ≥ 4%
- Risk estimate incorporates prior results (Pap + HPV)
- Tool designed for clinicians:
  - https://app.asccp.org/



Perkins RB, et al. J. Low. Gen. Tract. Dis. (2020) **24**: 102

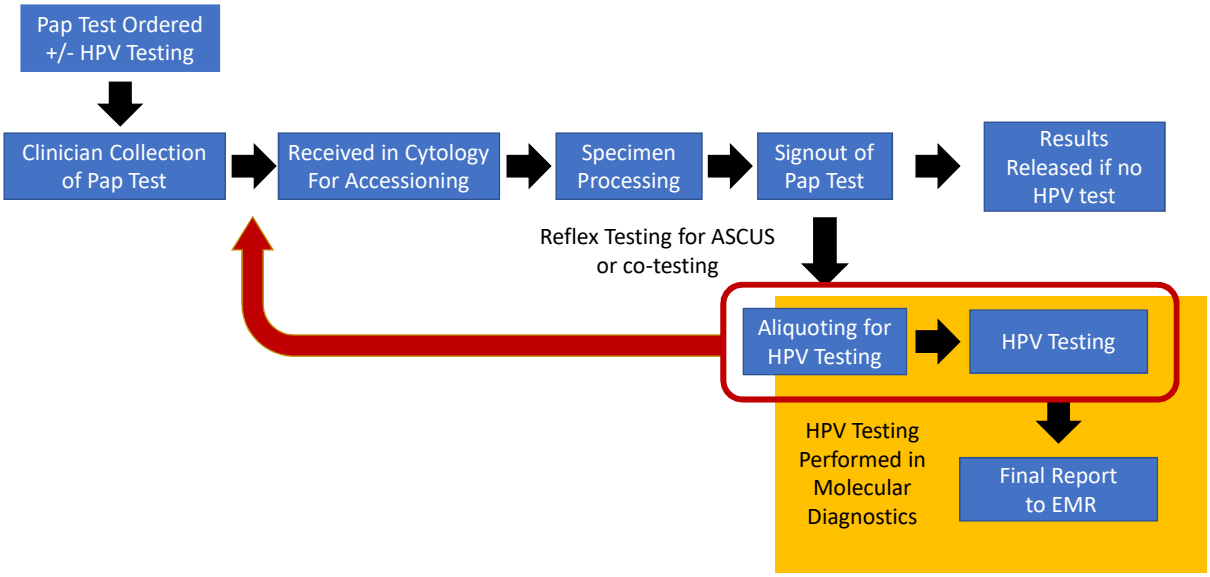
Primary HPV Screening in 2023



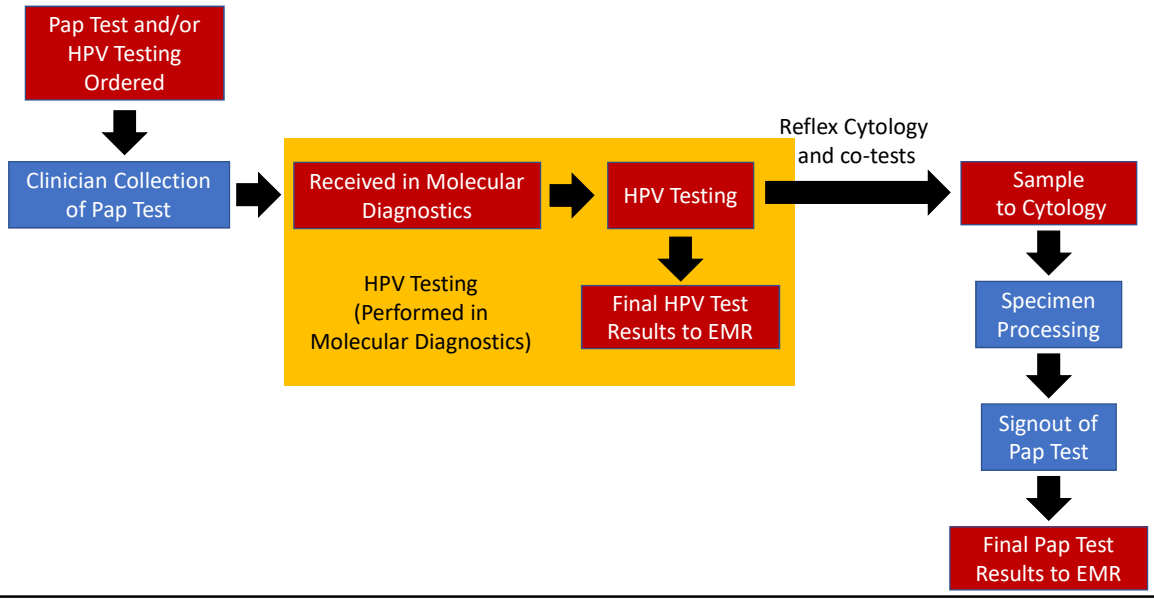
Triage of Abnormal Primary HPV Results

- Pair a highly sensitive test (primary HPV screening) with a more specific test
  - Genotyping: both FDA approved HPV assays for primary screening have genotyping for high-risk types 16 & 18 built in
  - Cytology: in the United States, cytology remains the dominant cervical cancer screening test

Modifying The Workflow

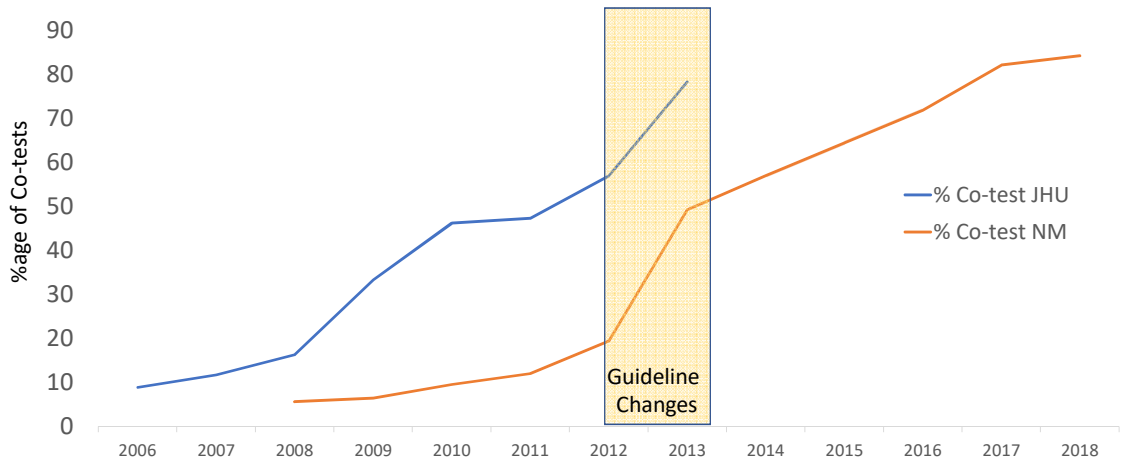


Modified HPV/GYN Cytology Workflow



What Will Happen to Pap Test Volumes???

Adoption of Co-testing



Cuzick J, et al. Gyn Onc (2021) **162**: 555
Silver MI, et al. Can. Causes Con. (2018) **29**: 43

Limited Number of FDA Approved Assays

- Currently 5 FDA approved tests on the market (co-testing)
  - Cervista (Hologic)
  - Hybrid Capture II (Qiagen)
  - Aptima (Hologic)
  - Cobas (Roche)
  - BD Onclarity (Becton Dickinson)
- Only 2 FDA approved assays for primary HPV screening:
  - Cobas (Roche)
  - BD Onclarity (Becton Dickinson)

Cervical Cancer Screening Practices

- Yabroff (2009): <25% of physicians reported guideline-consistent care
  - Varied by specialty: Internists more likely than family physicians, followed by gynecologists
  - Overuse of screening most common deviation
- Teoh (2015): 6% of clinicians utilized guideline-consistent care, 80% of clinicians responded correctly to the majority of situations
- Min (2020): only 2% of clinicians appropriately utilized guidelines in all situations
  - Overuse of screening (<21 y/o, >65 y/o)
  - Overtreat of persistent LSIL
  - Undertreat young patients with HSIL
- Vadaparampil (2023): 28-36% of participants were guideline adherent
  - >50% of these providers thought they were guideline-consistent with their care

Yabroff KR, et. al. Ann. Int. Med. (2009) **151**: 602 Min CJ, et. al. J. Low. Gen. Tract Dis. (2020) **24**: 337
Teoh DGK, e.t al. Am. J. Ob. Gyn. (2015) **212**: 62 Vadaparampil ET, et. al. Cancer (2023) May 23. epub

Patient Preferences

- Web-based survey of ~1300 women in the United States:
  - Primary HPV screening was the least accepted screening modality (13.5%)
  - Annual Pap test was the most accepted (41.2%)
  - Acceptance of primary HPV screening linked to participant knowledge of HPV
- Survey of 551 women aged 36-62:
  - 74% believed women should be screened annually
  - 68% would be willing to extend to q3 year screening if recommended by their physician
  - Only 25% would extend to 5 year screening interval
  - 61% expressed a strong preference for pap testing
  - 42% expressed at least moderate concern about HPV testing alone

Saraiya M, et al. Prev. Med. (2018) **108**: 111
Silver MI, et al. Obstet. Gyn. (2015) **125**: 317

Adoption of Primary HPV Screening

- Widespread shift to primary HPV screening will probably not take place until additional guidelines emerge (USPSTF)
- Availability of FDA approved assays
- Practice habits and patient preferences may trail guideline changes

Are There Unintended Consequences?

HPV "Negative" Lesions

Test	Hybrid Capture II	Aptima	Cobas	BD Onclarity
Sensitivity for CIN2/3	63.6%-100%	55.3%-100%	71.1%-99%	85.7%-100%
Specificity for CIN2/3	6.2%-98.4%	28.8%-99.2%	24%-86.2%	17%-98.8%

- Several studies have demonstrated significant numbers of HPV negative lesions:
  - Ge (Cobas): 8.3% of women with biopsy proven HSIL had preceding –HR HPV testing
  - Zheng (HC2): HPV testing was negative in 7.5% of patients in the year before an invasive cancer diagnosis
  - Zhao (HC2, Cervista, Cobas): 17% of pts with invasive carcinoma had a negative HPV test in the prior 5 years.

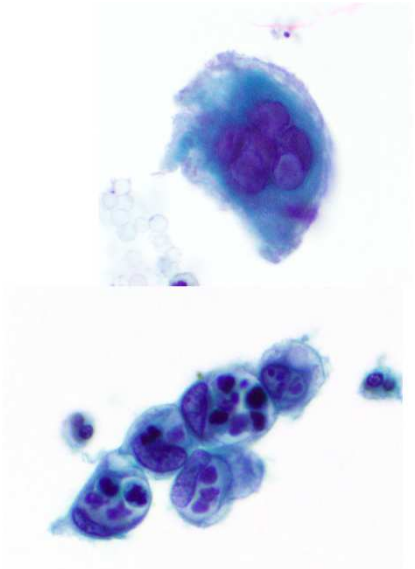
Ge Y, et al. JASC (2019) **8**: 149
Zheng B, et al. Can Cyto (2015) **123**: 428
Zhao C, et al. Arch Path & Lab Med (2014) **139**: 184

HPV "Negative" Lesions

- False negative HPV results
  - Bloody samples
  - Cellularity (β-globin)
  - Interfering substances
  - Less common HPV types

Non-HPV Driven Pathology

- Subset of STIs
- Pap test is reasonably sensitive for endometrial neoplasia
  - 45% with an abnormality on pap



Frias-Gomez J, et al. Cancer Cyto. (2020) **128**: 792

Knowledge of HPV Results Impacts Interpretation

- Cytotechnologist
  - Abnormal HPV result reduces NILM interpretations: ~10-17%
    - ⬆ Referral rate
- Pathologist:
  - Alters the usage of ASCUS
    - Upgrade: ~9%
    - Downgrade: ~29-34%

Concern for false negatives: bloody samples, interfering substances

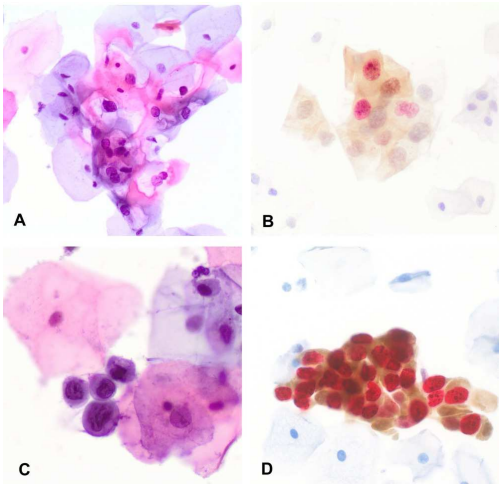
Doxtader EE, et al. Can. Cytopath. (2017) **125:** 60
Moriarty A, et. al. Arch. Path. Lab. Med. (2014) **138:** 1182
Aitken CA, et al. J. Med. Screen (2019) **26**: 221
Wright TC, et. al. AJCP (2016) **146:** 391

Triage of Abnormal Primary HPV Results

- Pair a highly sensitive test (primary HPV screening) with a more specific test
  - Genotyping: both FDA approved HPV assays for primary screening have genotyping for high-risk types 16 & 18 built in
  - Cytology: in the United States, cytology remains the dominant cervical cancer screening test
  - Future options:
    - Dual stain
    - Additional molecular testing: extended genotyping, methylation testing

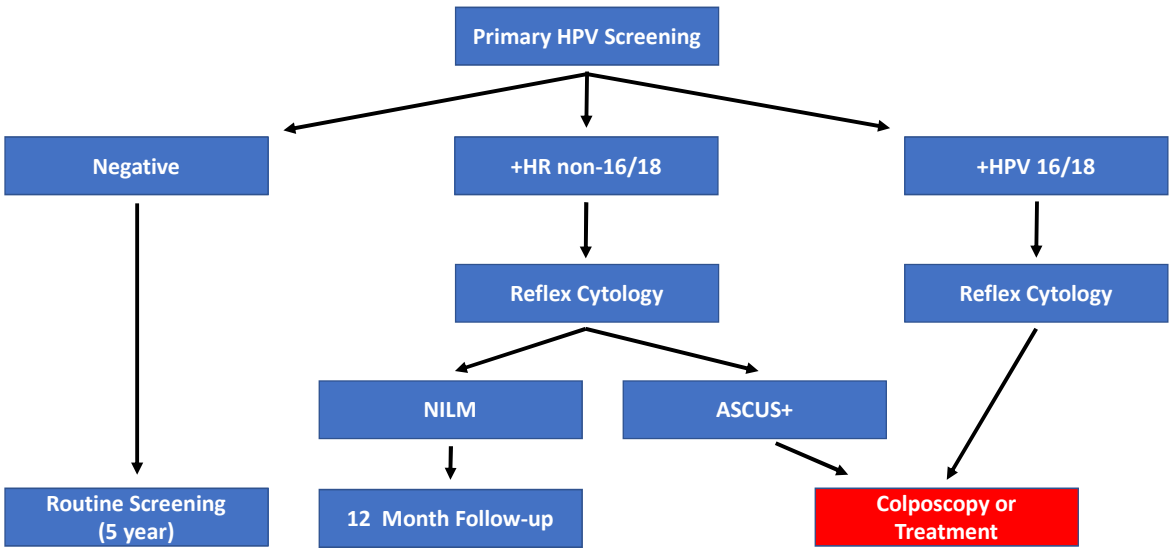
Dual Stain – p16/Ki-67

- Highly sensitive (90%) and specific (72%) for HSIL+
- Prospective study of 1549 HPV+ patients
  - ANY dual stain positive cells were associated with higher CIN2+ risk compared to ASCUS+ cytology (31% vs 25%, p=0.03)
  - Dual stain negative patients had significantly lower risks of CIN2+ compared to NILM cytology (8.5% vs 12.3%, p=0.04)



Ordi J, et. al. Can. Cyto. (2014) **122**: 227
Clark MA, et. al. JAMA Onc. (2019) **5**: 181

Primary HPV Screening in 2023



Summary

- Pap test was the basis for the most successful cancer screening program in history
- HPV testing is moving to the forefront in cervical cancer screening, but has limited specificity
- Widespread adoption of co-testing took place over several years, primary HPV screening may have a similar trajectory
- Adoption of primary HPV screening may necessitate extensive workflow and instrumentation changes
- Cytology remains the best positioned triage test in the US; however, results are biased when HPV results are known
- Additional triaging methods are on the horizon

Primary HPV Screening Abroad

- Primary screening modality in: Australia, The Netherlands, Turkey
- The Netherlands (2017):
  - Ages 30-60, q5 yrs until age 40 (q10 yrs)
  - Triage: Cytology triage
- Turkey (2014):
  - Ages 30-65, q5 yrs
  - Triage: Cytology and genotyping
- Australia (2017):
  - Ages 25-74, q5 yrs
  - Triage: Cytology and genotyping