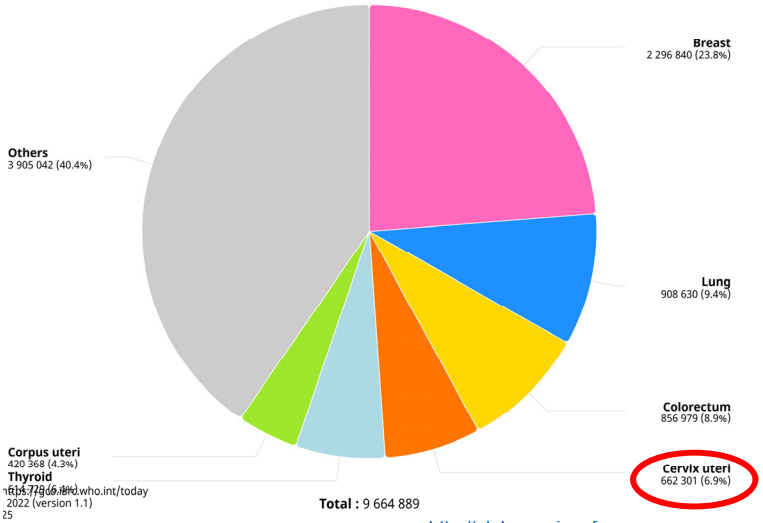
Integrating Primary HPV Screening into the Cytology Laboratory

Jeffrey Mito, MD, PhD
Brigham and Women's Hospital
Harvard Medical School

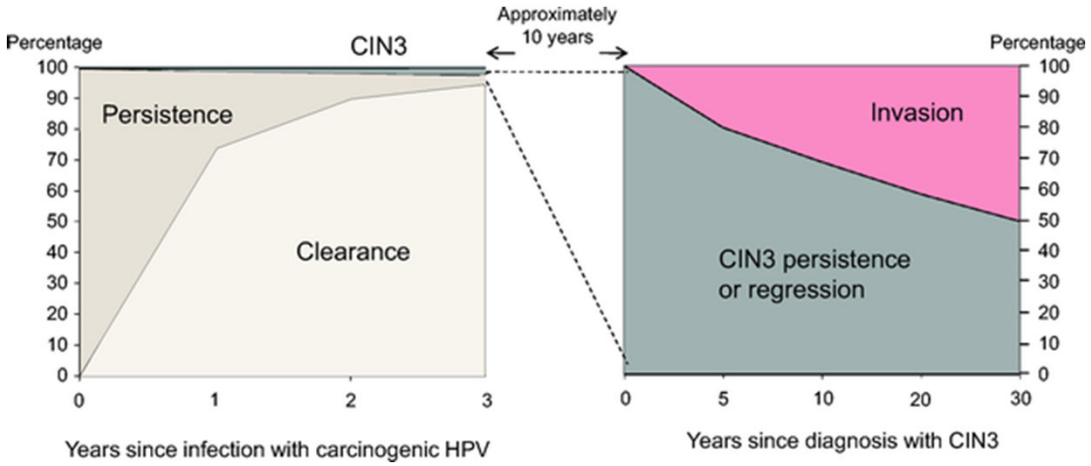
Cervical Cancer Incidence - 2022

- Common worldwide:
  - >660,000 cases
  - >340,000 deaths
- United States:
  - ~13,300 cases
  - ~4,300 deaths



http://globocan.iarc.fr
https://seer.cancer.gov/statfacts/html/cervix.html

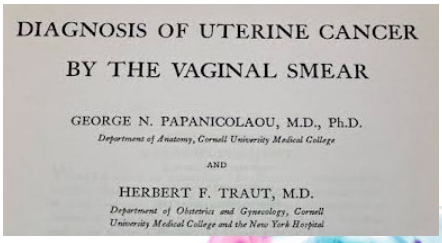
Development of Invasive Carcinoma



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
- Identified cancerous cells in the late 1920s
- Descriptive atlas of cells in vaginal smears
- 193 invasive carcinomas
  - All but 11 cases had "malignant" cells identified by vaginal smear
- First mass effort in early cancer detection (ACS, 1945)

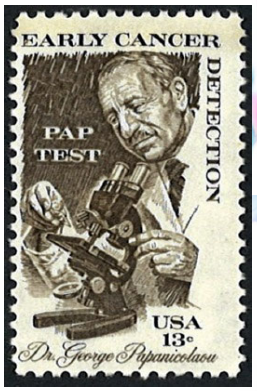


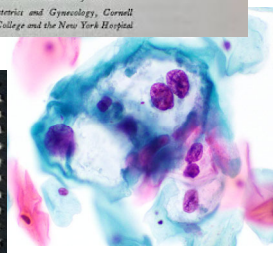
DIAGNOSIS OF UTERINE CANCER
BY THE VAGINAL SMEAR

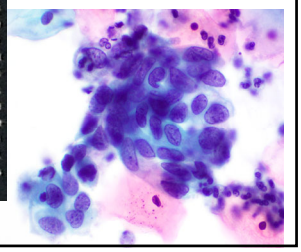
GEORGE N. PAPANICOLAOU, M.D., Ph.D.
Department of Anatomy, Cornell University Medical College

AND

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

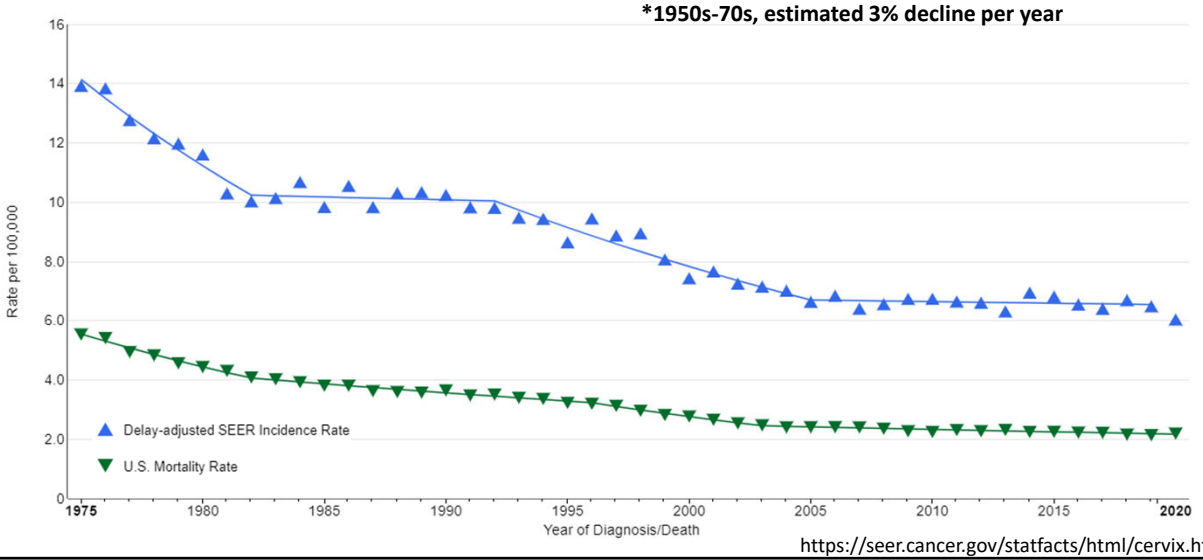






Longterm Trends in Cervical Cancer Incidence

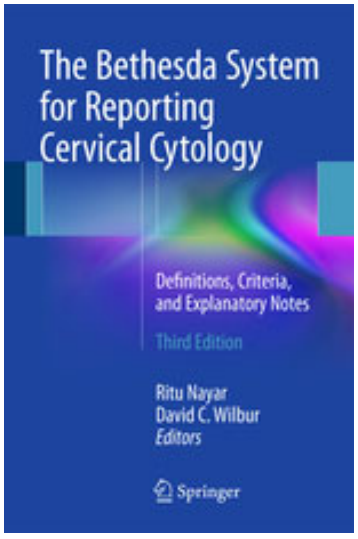
*** ~36 per 100,000 women in 1930s**
***1950s-70s, estimated 3% decline per year**



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

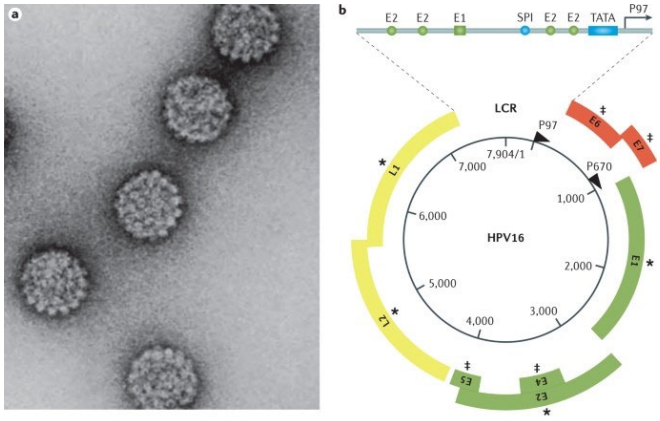
The Bethesda System

- 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

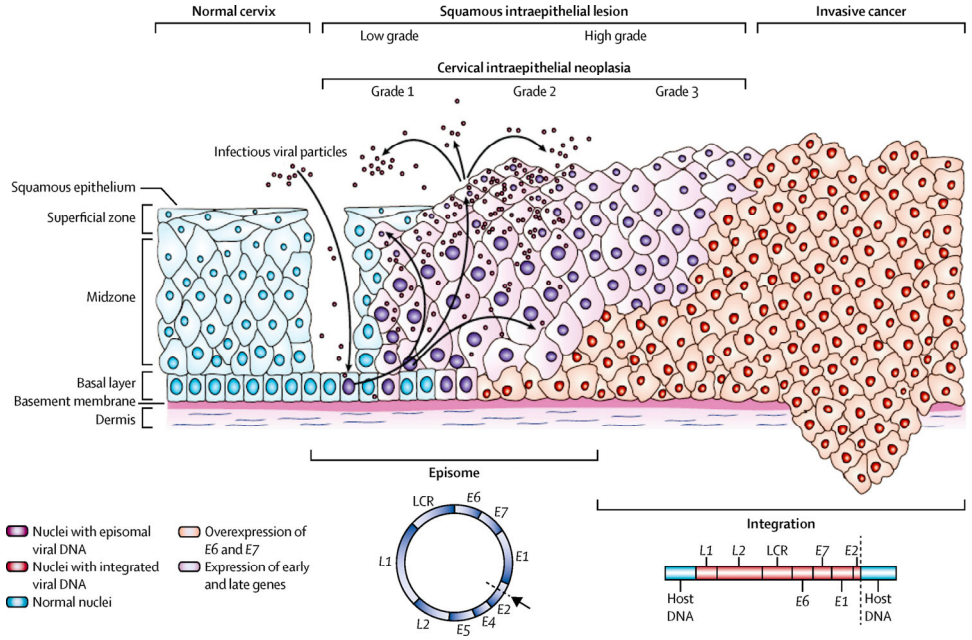


Human Papilloma Virus

- Most common sexually transmitted infection in the United States
- Linked to cervical cancer in 1974
- 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



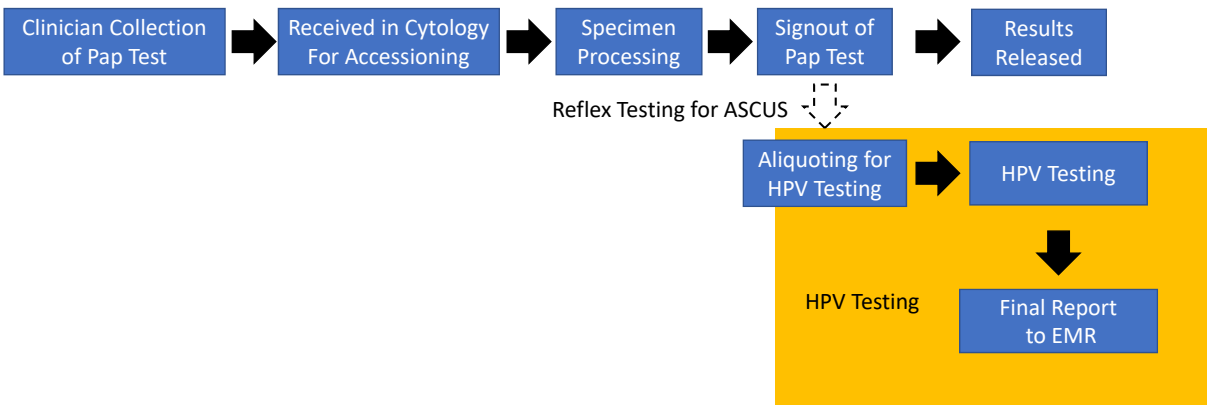
Cohen PA, et al. The Lancet (2019) **393**: 169

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: 1) immediate colposcopy, 2) repeat cytology, or 3) 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

Reflex HPV Testing Workflow

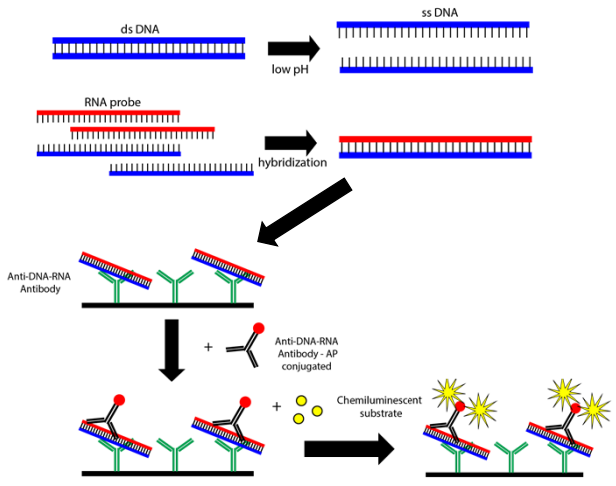


Test	Hybrid Capture II	Aptima	Cobas	BD Onclarity	Alinity m
Manufacturer	Qiagen	Hologic	Roche	Becton Dickinson	Abbot
FDA approved for reflex/co-testing	2001	2011	2011	2018	2023
Method	DNA (non-PCR) Signal amplification: full genome probe	**mRNA** *in vitro* transcription: E6/E7 gene target	**DNA** (qPCR based): L1 gene target	**DNA** (qPCR based): E6/E7 gene target	**DNA** (qPCR based): L1 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, 68	16*, 18*, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68	16*, 18*, 31*, 33, 35, 39, 45*, 51*, 52*, 56, 58, 59, 66, 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	Various
Sensitivity for CIN2/3	63.6-100%	55.3-100%	71.1-99%	85.7-100%	85.29-100%
Specificity for CIN2/3	6.2-98.4%	28.8-99.2%	24-86.2%	17-98.8%	54.9-92.4%
Built-in internal control	No	HPV16 E6/E7 transcript is added	Yes (ß-globin)	Yes (ß-globin)	Yes (ß-globin)

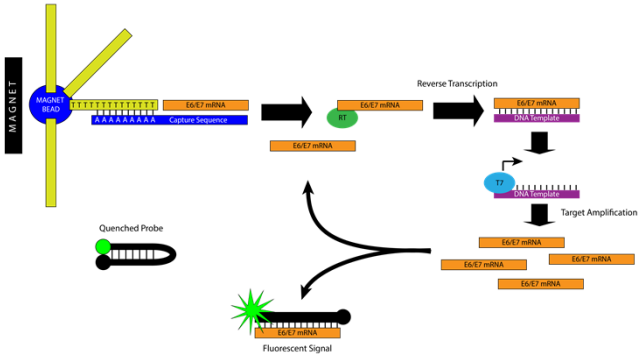
Modified from Salazar KL, et al. JASC (2019) **8**: 284

FDA Approved HPV Assays

Hybrid Capture 2

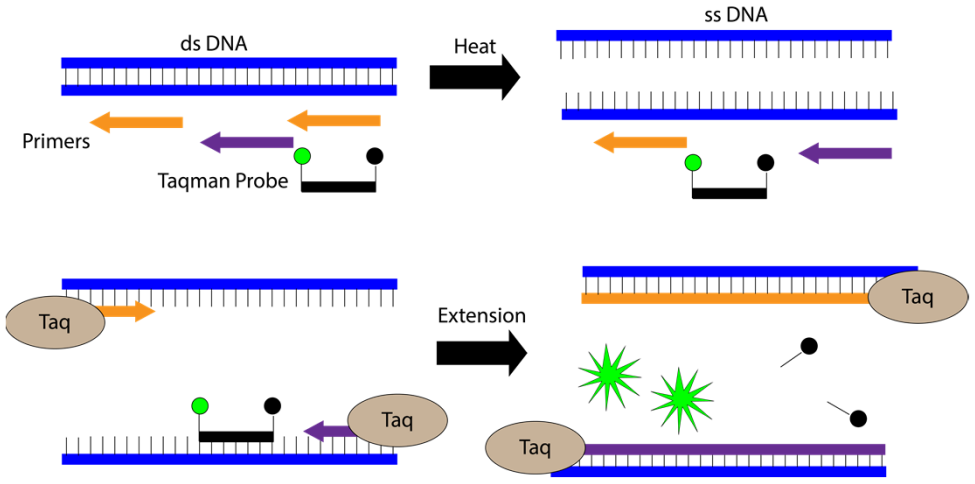


Aptima



FDA Approved HPV Assays

Alinity m/Cobas/Onclarity

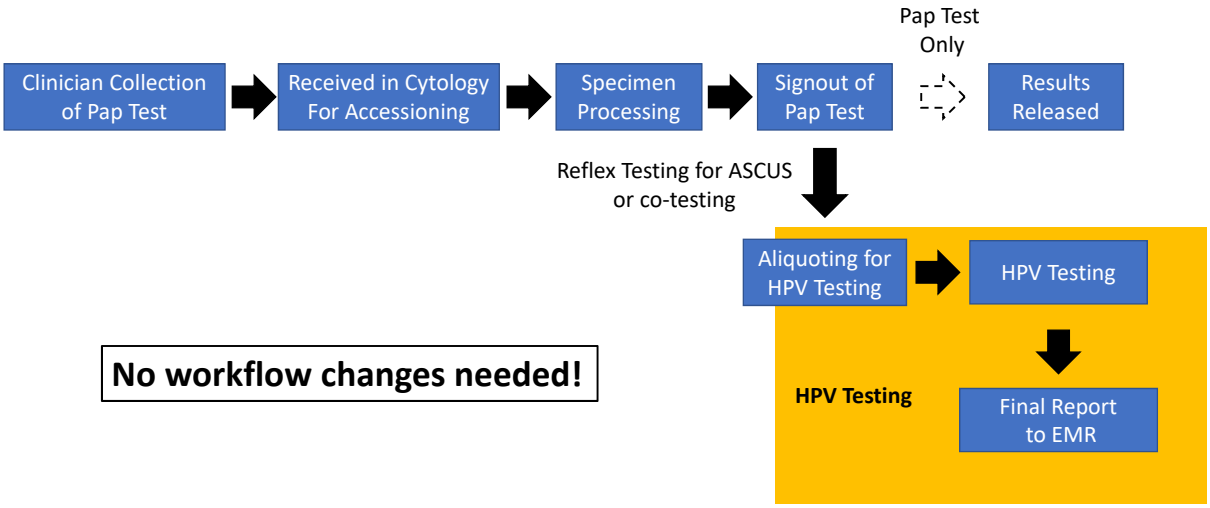


Co-Testing

- Pap test with HPV testing
- Increased overall sensitivity of the pap test
  - Increased detection of CIN3 in initial rounds of screening
  - Improved detection of endocervical adenocarcinoma
- 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
Saslow D, et al. CA Cancer J. Clin. (2012) **62**: 147

Gyn Co-testing 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

- Screening approaches need to be reconsidered with increasing number of HPV vaccinated individuals
- A negative HR-HPV test result has a lower cumulative incidence of CIN3+ than cytology at 3- or 5-year follow-up
- Primary HPV screening with triage using genotyping AND cytology increases detection of CIN3+ over cytology alone, which is predicted to prevent 1 additional case of invasive cancer per 1000 screened individuals over cytology alone
- Cytology alone fails to detect a significant portion of CIN3+ lesions in younger patients
- Reduced number of lifetime screenings as well as follow-up tests and colposcopies
- Option for self-collection

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]

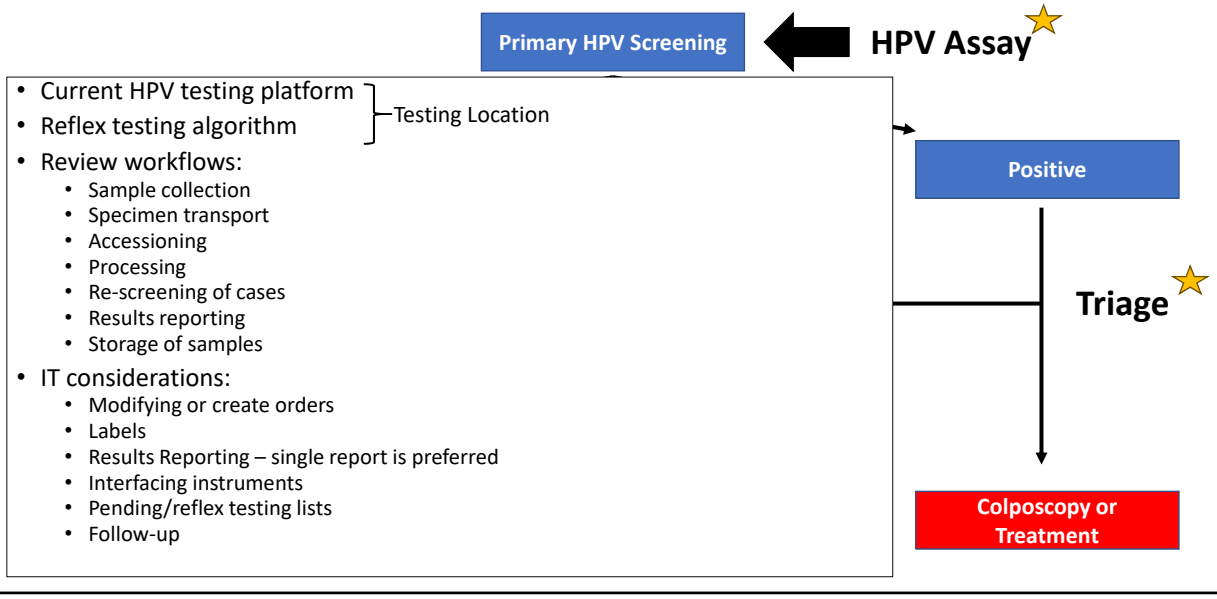
"The ACS now recommends primary HPV testing at a 5-year interval as the preferred screening strategy *for all individuals being screened*."

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

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

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

Implementing Primary HPV Screening

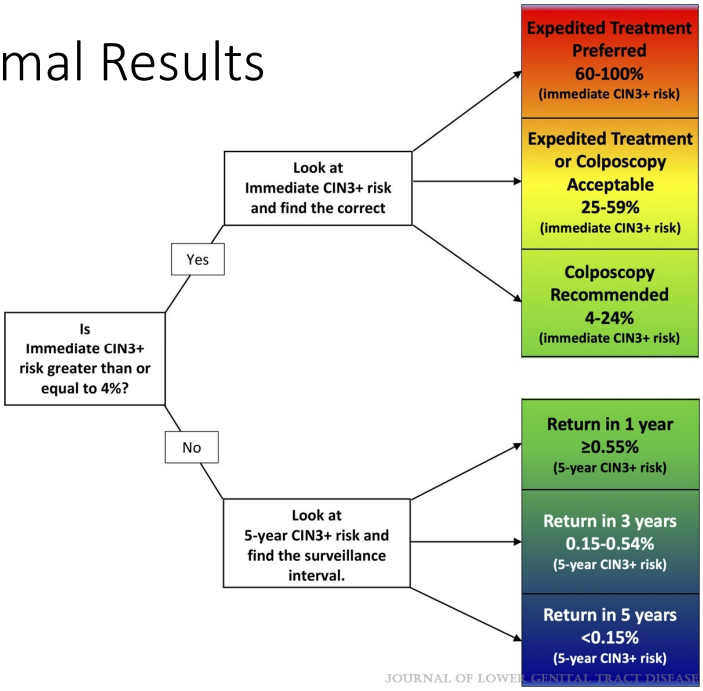


Test	Hybrid Capture II	Aptima	Cobas	BD Onclarity	Alinity m
Manufacturer	Qiagen	Hologic	Roche	Becton Dickinson	Abbot
FDA approved for reflex/co-testing	2001	2011	2011	2018	2023
FDA approved for primary screening	N/A	N/A	**2014 (ThinPrep)** **2018 (Surepath)**	**2018 (SurePath)** **2023 (ThinPrep)**	**2023 (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	**DNA** (qPCR based): L1 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	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	Various
Sensitivity for CIN2/3	63.6%-100%	55.3%-100%	71.1%-99%	85.7%-100%	85.29-100%
Specificity for CIN2/3	6.2%-98.4%	28.8%-99.2%	24%-86.2%	17%-98.8%	54.9-92.4%

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/



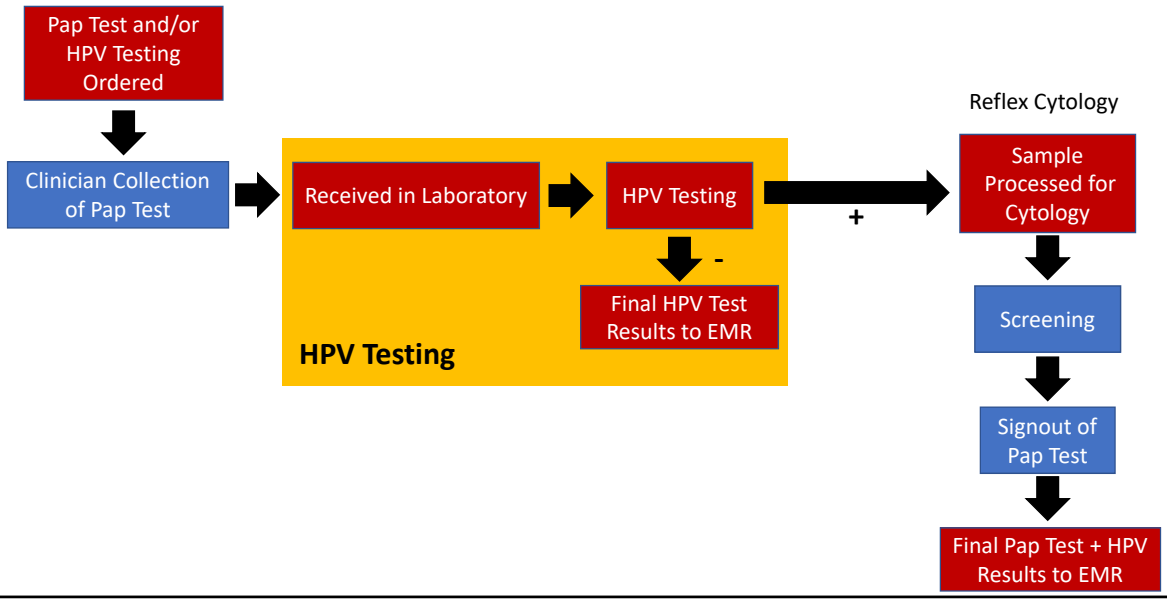
Perkins RB, et al. J. Low. Gen. Tract. Dis. (2020) **24**: 102

Triage of Abnormal Primary HPV Results

- Pair a highly sensitive test (primary HPV screening) with a more specific test
  - Cytology: in the United States, cytology remains the dominant cervical cancer screening test
  - Dual stain (p16/Ki-67)
  - Genotyping:
    - Partial or limited – HPV types 16/18 alone
    - Extended genotyping
  - Future: viral load or methylation testing

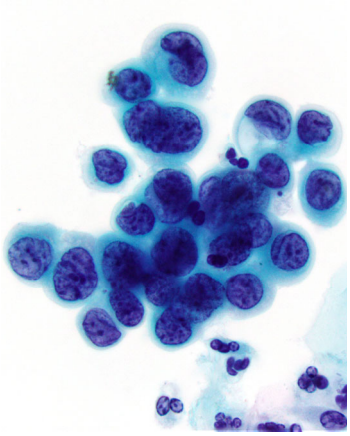
Thrall MJ, et al. JASC (2025) **14**: 11

Reflex Cytology Workflow



Reflex Cytology for HPV+ Results

- Advantages:
  - Widely available
  - Leverages existing workflows and expertise of Cytologists
  - Distinguish glandular and squamous lesions
  - FDA approved Digital Cytology option
- Disadvantages:
  - Labor intensive - projected declines in the workforce
  - Results are subject to sampling
  - Morphologic evaluation can be subjective
  - Knowledge of HPV results can influence interpretations
  - Not compatible with self-collection



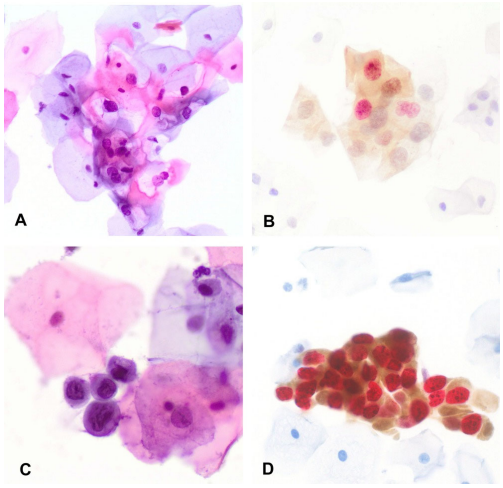
Knowledge of HPV Results Impacts Interpretation

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

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

Dual Stain – p16/Ki-67

- Targeting p16 (brown chromogen) and Ki-67 (red chromogen)
- FDA approved in 2020:
  - Triage of HPV positive individuals with or without limited genotyping
  - Triage of HPV positive results in conjunction with NILM cytology
- 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

Dual Stain Recommendations and Guidelines

- Only apply to FDA approved dual stain assays
- Recommended management:

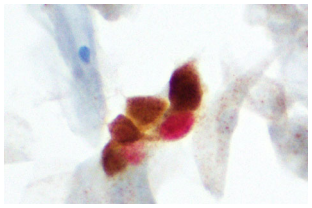
		HPV 16/18		HPV Other	
		16 +	18 +	+	All -
Dual Stain	+	Colposcopy	Colposcopy	Colposcopy	n/a
	-	Colposcopy*	Colposcopy*	1-year follow-up	n/a

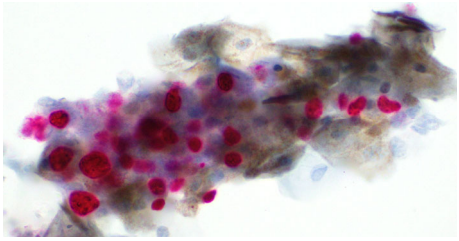
- If the dual stain is unsatisfactory due to sampling, and there is insufficient information for risk-based management, repeat testing should be performed no later than 4 months

Clarke M, et al. J. Low. Gen. Tract Dis. (2024) **28**: 124

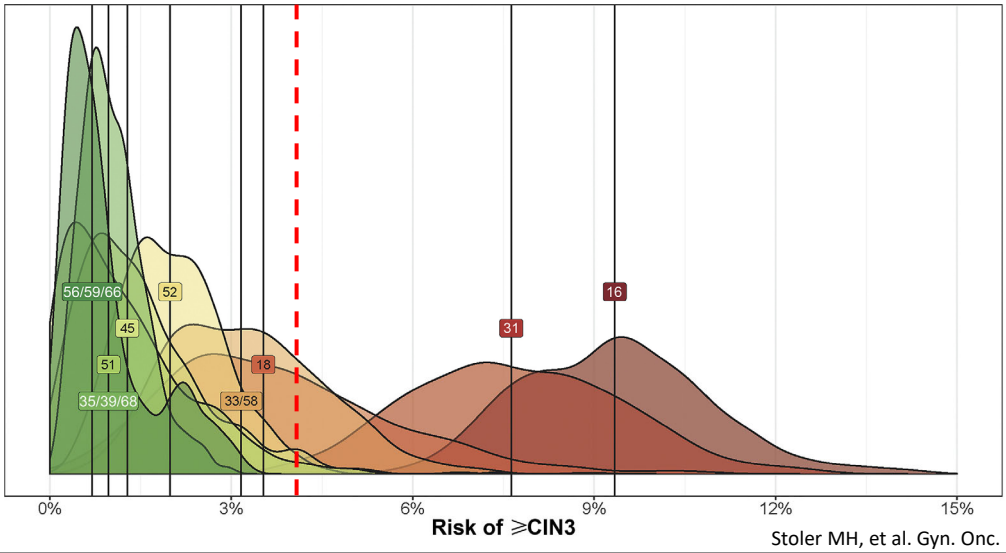
Dual Stain

- Advantages:
  - Greater sensitivity for CIN2+ than cytology when triaging primary HPV+ patients
  - Morphology based – leverage expertise of Cytologists
  - Potentially amenable to AI/digital cytology
  - Reimbursement
- Disadvantages:
  - Low throughput
  - Single FDA approved staining platform
  - Additional training – Pathologist and Cytologists
  - Requires pathologist review
  - Not compatible with self-collection
  - Reimbursement – difficulty with payers





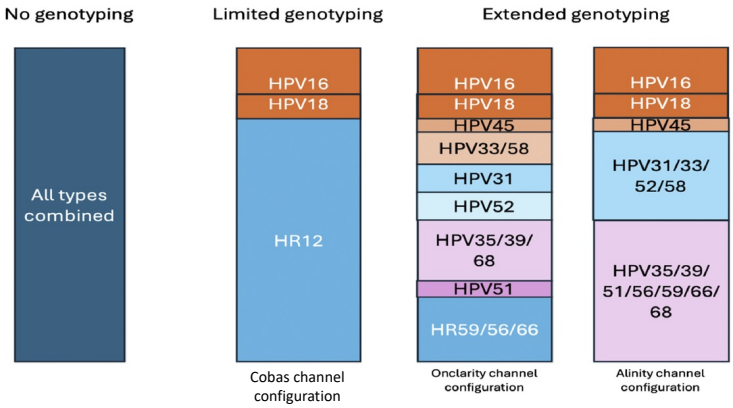
Risk of CIN3+ by HPV Genotype



Stoler MH, et al. Gyn. Onc. (2019) **153**: 26-33

Extended Genotyping Recommendations and Guidelines

- Recommendations only apply to FDA approved extended genotyping assays
- Can operate as a "stand alone" test or in conjunction with cytology or dual stain triage



Massad LS, et al. J. Low. Genit. Tract Dis. (2025) **29**: 134

	Current HPV	Current cytology	Past results	Management
HPV 16/18	16	HSIL[1]	N/A[2]	Treatment preferred; colposcopy acceptable
	16	ASC-H[3]	N/A	Treatment or colposcopy
	16	NILM,[4] ASC-US,[5] LSIL,[6] AGC[7], or no cytology	N/A	Colposcopy[8] *with collection of cytology if not already done*
	18	HSIL	N/A	Treatment or colposcopy
	18	NILM, ASCUS, LSIL, ASC-H, AGC, or no cytology	N/A	Colposcopy[8] *with collection of cytology if not already done*
HPV 45,33/58, 31, 52/35/39/68, 51 **Untyped or "other" types when 16 and 18 are not present**	45,33/58, 31, 52/35/39/68, 51 or untyped/other	HSIL, ASC-H, AGC	N/A	Colposcopy[8,9]
	45,33/58, 31, 52/35/39/68, 51	ASC-US or LSIL	N/A	Colposcopy
	Untyped/other	ASC-US or LSIL	Documented HPV negative screen in past 5 years or colposcopy <CIN2[10] in past year	Repeat HPV test in 1 year
	Untyped/other	ASC-US or LSIL	Any history other than above	Colposcopy
	45,33/58, 31, 52/35/39/68, 51 or untyped/other	NILM	Normal[11] or colposcopy <CIN2 within past year	Repeat HPV test in 1 year
	45,33/58, 31, 52/35/39/68, 51 or untyped/other	N/A	HPV+ without colposcopy (i.e. current test is 2nd consecutive HPV+)	Colposcopy
HPV 59/56/66	59/56/66	ASC-H, AGC, or HSIL[12]	N/A	Colposcopy[8]
	59/56/66	NILM, ASC-US, LSIL or no cytology[12]	Normal or colposcopy <CIN2 within past 1 year	Repeat HPV test in 1 year
	59/56/66	N/A	HPV+ without colposcopy (i.e. current test is 2nd consecutive HPV+)	Colposcopy

Massad LS, et al. J. Low. Genit. Tract Dis. (2025) **29**: 134

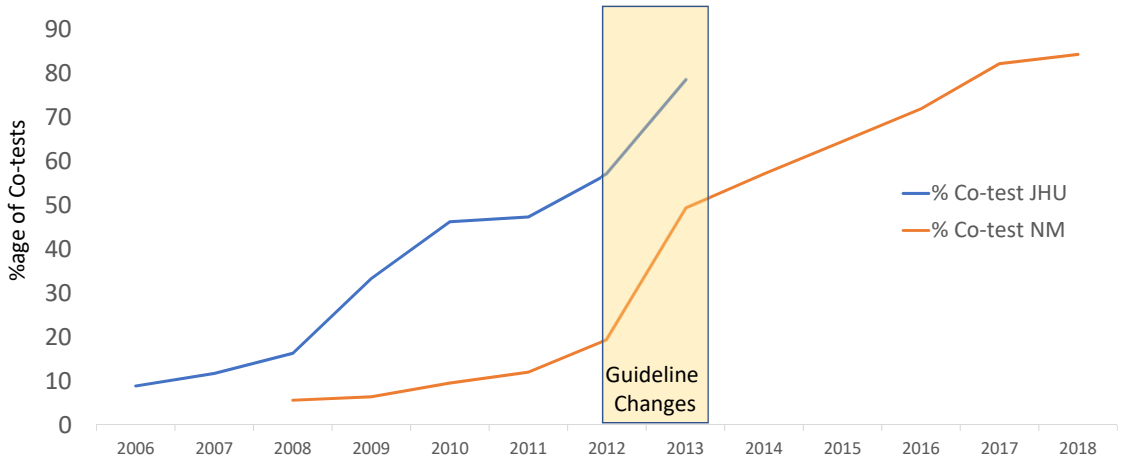
Extended Genotyping

- Advantages
  - Compatible with self-collection
  - Highly sensitive
  - Minimal additional cost
  - Can be integrated into workflow with other triage tests
- Disadvantages
  - No morphologic evaluation
  - Limited number of FDA approved assays
  - Multiple subgroups for risk and limited clinical data in absence of additional tests (Pap test or dual stain)
  - Potential increased number of colposcopies

Adoption of Primary HPV Screening

- In the United States, widespread clinical “demand” for primary HPV screening will probably not take place until USPSTF recommendations are finalized
- Availability of FDA approved assays
- Practice habits and patient preferences may trail guideline changes

Adoption of Co-testing



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

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

Self-Collected Vaginal Samples for HPV Testing

- Two FDA approved assays: Onclarity and Cobas
- Not compatible with cytology or dual stain
- ASCCP Recommendations:
  - Self-collected vaginal samples are *acceptable* for cervical cancer screening
  - Use of an FDA approved collection kit and assay
  - *HPV- samples should have repeat testing in 3 years*
  - Triage of abnormal results:
    - HPV 16/18 + ⟹ refer to colposcopy with concurrent Pap test
    - Other HPV results* ⟹ Follow-up Pap test or dual stain

      *Except HPV types 56/59/66: 1 year repeat testing
- Most patients prefer self-sampling compared to samples obtained by a healthcare provider (51-93%)

Nicolas W, et al. J Low. Gen. Tract Dis. (2025) **29**: 144
Morgan K, et al. J Low. Gen. Tract Dis. (2019) **23**: 193

HPV "Negative" Lesions

Test	Cobas	BD Onclarity	Alinity m
Sensitivity for CIN2/3	71.1%-99%	85.7%-100%	85.29-100%
Specificity for CIN2/3	24%-86.2%	17%-98.8%	54.9-92.4%

- 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

Significant outcomes associated with high-risk human papillomavirus negative Papanicolaou tests

Selda Karaaslan, MD, PhD, Thomas L. Dilcher, BS, Mary Abdelsayed, CT (ASCP), Abha Goyal, MD*

Department of Pathology and Laboratory Medicine, Weill Cornell Medicine-New York Presbyterian Hospital, New York, New York

- ~2500 ≥ ASC-H Pap test samples with concurrent HPV results
- ~30% of co-test samples diagnosed as ≥ ASC-H were HPV negative

Karaaslan S, et al. JASC (2023) **12**: 189

HPV Negative Pap Tests

Table 1 Papanicolaou Test Categories included in the Study Cohort with their Concurrent High-Risk Human Papillomavirus Test Results.

Pap test category	Number of cases	Number of HPV tested cases (%)	Number of HPV negative cases (%)	Number of HPV-negative patients included in final study cohort
CA	26	22 (84.6)	7 (31.8)	7
SUSP	27	15 (55.5)	2 (13.3)	1
HSIL	1050	795 (75.7)	73 (9.2)	65
ASC-H	1074	888 (82.7)	291 (32.8)	263
LSIL-H	587	391 (66.6)	60 (15.3)	54
AEM	134	96 (71.6)	82 (85.4)	82
AGC, NOS	290	207 (71.4)	164 (79.2)	161
AGCFN	20	14 (70.0)	13 (92.8)	13
AEC, NOS	149	131 (87.9)	118 (90.0)	115
AECFN	14	3 (21.4)	2 (66.7)	2
AIS	2	2 (100)	0	0
Total	3373	2564 (76.0)	812 (31.7)	763

Karaaslan S, et al. JASC (2023) **12**: 189

Follow-up of HPV Negative Cases

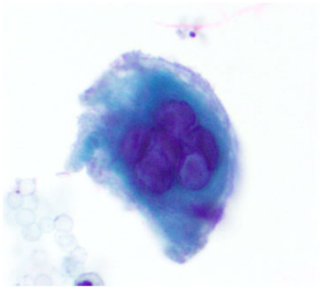
Table 2 Histologic follow-up of patients with negative high-risk human papillomavirus test result and squamous cell abnormalities on the Papanicolaou test.

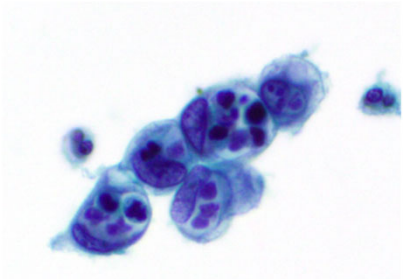
Pap test category	Study cases	Cases with follow-up (%)	Significant findings (number of patients)	Percentage of patients with follow-up with significant findings
Squamous cell carcinoma	4	2 (50.0)	At least CIN 3 (1), Metastatic squamous cell carcinoma (1)	100.0
SUSP	1	1 (100)	CIN 3 (1)	100.0
HSIL	65	58 (89.2)	CA (1), CIN 3 (13), CIN 2 (9)	39.6
ASC-H	263	189 (71.9)	CIN 2 (9), CIN 3 (9)	9.5
LSIL-H	54	39 (72.2)	CIN 2 (6), CIN 3 (2)	20.5
Total	387	289 (74.7)	52	17.9

Karaaslan S, et al. JASC (2023) **12**: 189

HPV "Negative" Lesions

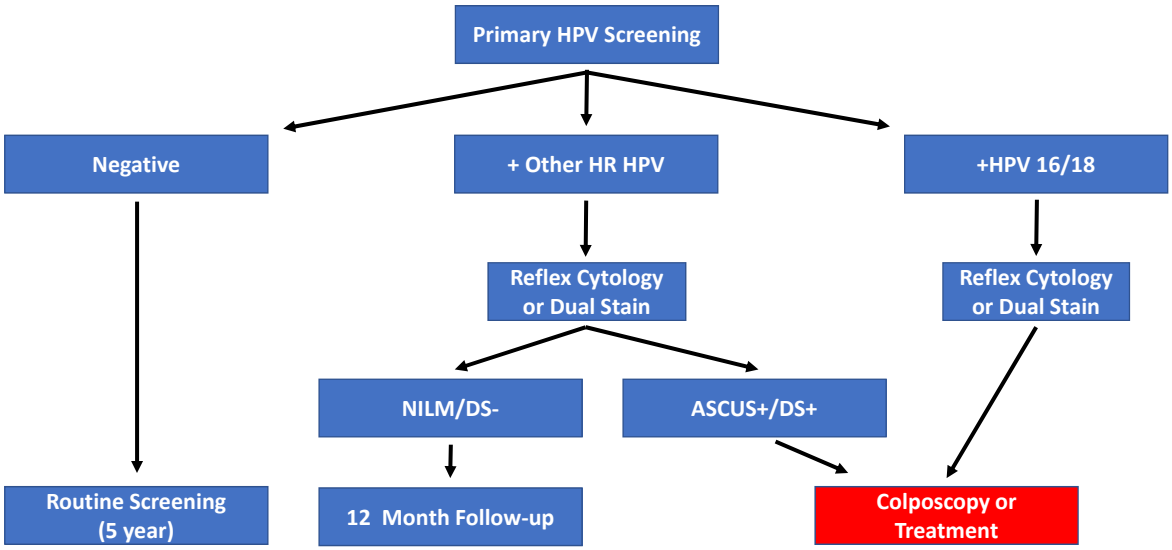
- False negative HPV results
  - Bloody samples
  - Cellularity (β-globin)
  - Interfering substances
  - Less common HPV types
- Truly HPV independent lesions
- Pap test is reasonably sensitive for endometrial neoplasia
  - 45% with an abnormality on pap
- Subset of STIs





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

Primary HPV Screening in 2025



Summary

- HPV based testing is at the forefront in cervical cancer screening, but has limited specificity as a standalone assay
- Adoption of primary HPV screening may necessitate extensive workflow and instrumentation changes
- Cytology remains the best positioned triage test in the US
- Additional triaging methods are available, but have barriers to rapid implementation