HPV Testing, From Triage to Screening and Prognosis (Head and Neck): Where are we today and where do we go from here ?

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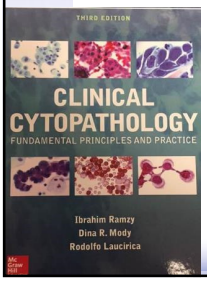
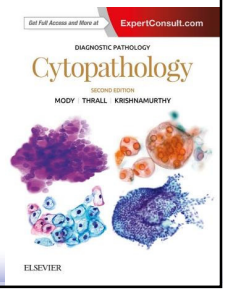
Conflict of Interest

- None with vendors of cytology equipment or HPV testing
- Amirsys (now Elsevier) and McGraw Hill
  - (Book publishers/Royalties)

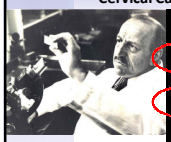


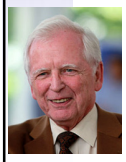


For This Talk……

- Historical perspective
- HPV facts, testing types, Issues
- Current Role of HPV testing in cervicovaginal cytology
  - HPV testing as triage
  - HPV co-testing
  - HPV as primary screen
  - HPV for QA/benchmarking
  - Risk Stratification and Guidelines
- HPV testing in head and Neck cancers (CAP guidelines)

Cervical Cancer Screening Milestones



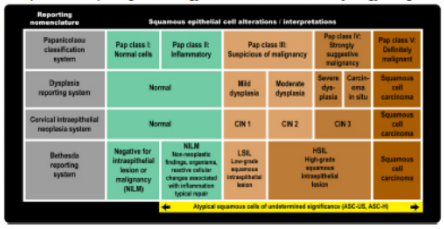


1914	Dr. George Papanicolaou emigrates to USA and starts research at Cornell
1928	Papanicolaou publishes findings on detection of cancer cells using vaginal smears in humans
1943	Papanocolaou and Traut monograph
1954	American Cancer Society endorses Pap test
1960	Pap smear widely available in USA
1965	First reports characterizing double-stranded HPV virus
1974	zur Hausen publishes link between cervical cancer and HPV
1976	Meisels and Fortin postulate koilocytes are viral cytopathic effect of HPV infection
1983	HPV 16, 18 detection in cervical cancer by Dr. zur Hausen
1988	CLIA '88 cytology mandates and first Bethesda conference for cytology reporting
1996	ALTS trial (USA) with Hybrid capture II (Digene)
2000	
2006	FDA approves first Prophylactic HPV vaccine (Gardasil 4)
2008	Dr. zur Hausen bestowed Nobel prize for medicine
2014	FDA approval of cobas platform (Roche) for primary HPV screening
2017	Implementation of primary HPV screening in some countries

Graphic From Diagnostic Pathology cytopathology 2nd ed by Mody

Evolution of Cervicovaginal Cytology Reporting Terminology

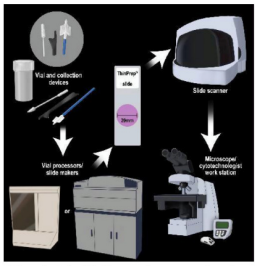
Comparison of Reporting Terminology for Normal and Abnormal Cytology Categories



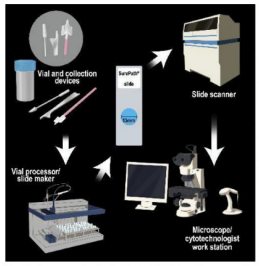
The cervicovaginal cytology reporting changed over a 60-year period, starting with Dr. Papanicolaou's classes. Pap classes I & II correspond to normal in the dysplasia and CIN reporting and NILM under The Bethesda System (TBS). Although the first 3 systems are linear, TBS is nonlinear. It introduced atypical squamous cells, which can include changes from benign to the neoplastic spectrum. Preneoplastic and neoplastic reporting are shown in various shades of increasing severity.

From 2nd edition of Diagnostic Pathology Cytopathology eds Mody Thrall Krishnamurthy Elsevier, Manitoba, 2018

CYTOPREPARATION, INSTRUMENTATION, AND AUTOMATED SCREENING IN GYNECOLOGIC CYTOLOGY



A flow chart for ThinPrep specimen processing shows the equipment needed to collect the cervicovaginal specimen, concentrate cells from the vial onto a slide, and perform imager-assisted screening.



SurePath processing is similar to ThinPrep, but note the smaller area of cells on the slide and the monitor for visual display of imager fields of view. The slide scanner can also accommodate conventional smears.

From chapter by M J Thrall in Diagnostic Pathology: Cytopathology Mody: Amirsys/Elsevier 2014, 2018

HPV Facts

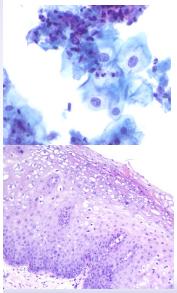
- Over 200 genotypes
- 40 anogenital genotypes
- Most genital HPV types occupy a single genus with 15 species (L1 capsid gene)
- Genotypes from same species share 60% or > nucleotide sequences
- Hence similar biological and pathological properties

HPV Facts…continued

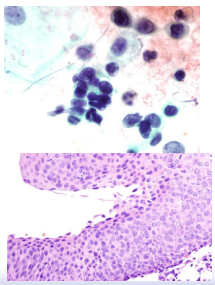
- The 13 high risk HPV types belong to 2 species (α-7 or α-9)
- Order of frequency worldwide: 16,18, 58,33,45,31,52,35,59,39,51,56
- Types 66 and 68 added later

Types of HPV Infection

Episomal

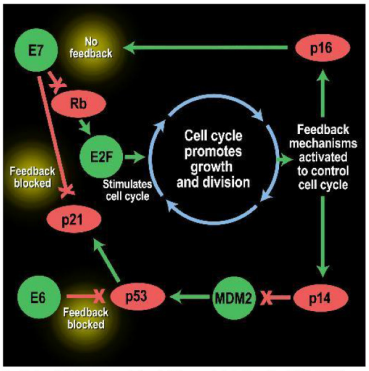


Integrated



HPV AND OTHER MOLECULAR TESTING IN GYNECOLOGIC CYTOLOGY

Chapter by Thrall MJ
Diagnostic Pathology
Cytopathology
Mody DR
Amirsys
2014



Simplified graphic highlights key mechanisms that drive uncontrolled cell growth and division in HPV-infected cells. HPV early protein, E7, stimulates cell cycle by binding to Rb and releasing E2F. This in turn

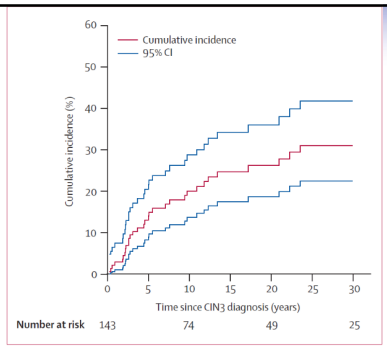
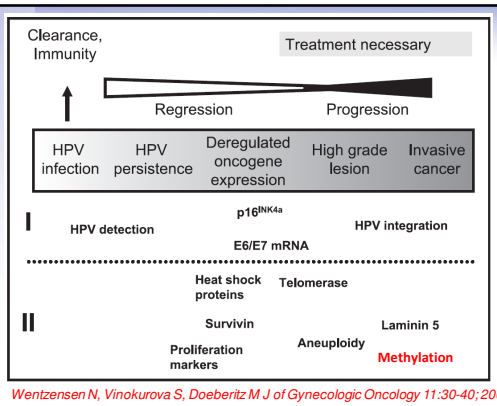
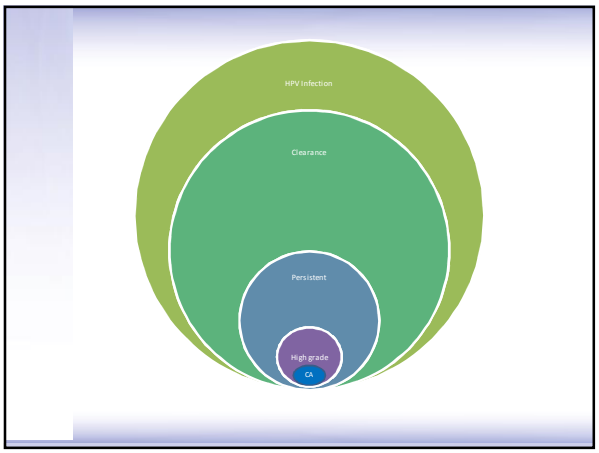


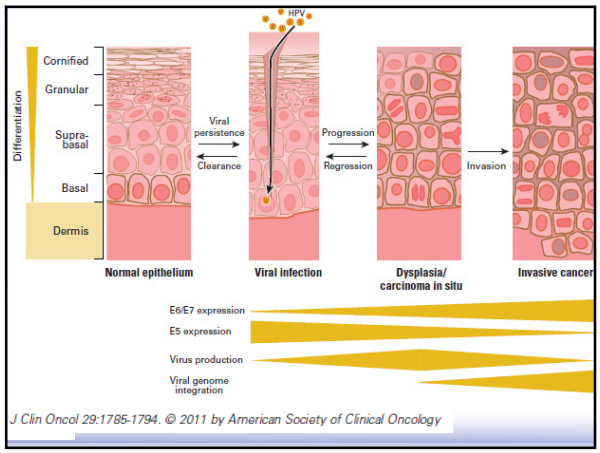
Figure 3: Cumulative incidence of cancer of the cervix or vaginal vault in women with minimum disturbance of the CIN3 lesion (no more than a punch or wedge biopsy)

Ref: Natural history of cervical neoplasia and risk of invasive cancer in women with CIN3; a retrospective cohort study by McCredie M, Sharples K, Paul C et al. Lancet Oncology 2008; (9):425-34



Wentzensen N, Vinokurova S, Doeberitz M J of Gynecologic Oncology 11:30-40; 2006





Types of FDA Approved HPV tests for Cytology in USA

Test	Manufacturer	Year Approved	Method	Number of HR Types
HCII	Qiagen	2001	DNA*	13. No genotyping
Cervista	Hologic	2009	DNA	14 and genotyping
Cobas	Roche	2011	DNA	16,18+12 types
Aptima**(Panther , Tomcat, Gemini)	Gen Probe	2011	RNA(E7mRNA)	16,18 + 12 types
Onclarity(Viper system)	BD	Feb 2018	DNA	16, 18+/- 45,31,51,52 and 6 other types

*No internal control
** Hologic purchased in Aug 2012

HPV Probes

	High-risk HPV	Non High-risk HPV
Hybrid Capture 2 HPV DNA test	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68	cross reactivity with 6, 11, 42, 43, 44, 53, and others
Cervista HPV HR test	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68	Limited cross reactivity: 67 and 70 (unknown risk types)
Cobas 4800 HPV test	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 16,18,non 18/18 genotypes	Limited cross reactivity: 6, 42, 54, 55, 62, 89
Aptima HPV assay	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 16,18/45 and other 11 genotypes	low-risk HPV genotypes 26, 67, 70, and 82
BD Onclarity	16,18,31,33,35,39,45,51,52 56,58,59,66,68 Genotypes 16,18,31,45,51,52 individually, 56,59,66 and 35,39,68 and 33,58 in pools	Data not available yet…

Part of Slide courtesy Dr N Nelles, Resident in Pathology, Methodist Houston

Sensitivity, Specificity & PPV of Various HPV tests for High Grade Disease (CIN 2+)*Predictors 2 study

Test	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)
Qiagen Hybrid capture 2	96.3	19.5	37.4
Roche Cobas	95.2	24	37.6
Gen-Probe Aptima	95.3	28.8	39.3
BD Onclarity	95	24.2	37.8
P16 on cytology	85.7	54.7	49.1
Cytology (CIN1 or>)	88.9	58.1	50.7

*Based on referral pap being borderline (ASCUS) or LSIL/low grade
*Not general screening population

Szarewski A et al. *J of clin microbiology* 2012;50(6):1867-73

HPV False Negative Results at the time of Cervical Cancer Diagnosis

Reference	Country	# Cervical Cancers	Collection Vial	# + HC2	NegHC2 (%)
JClinVirol 2006 35:264-269	China	475	STM	427	48 (10.1%)
IntJGynCa 2009 19:924-928	Korea	198	STM	185	13 (6.6%)
IntJGynCa 2006 16: 586-590	Brazil	168	STM	148	20 (11.9%)
ActaDematoven APA 2009 18: 940-103	Slovenia	95	STM	83	12 (12.6%)
	Total	936		843	93 (9.9%)

CIN2+ Sensitivity HC2 = Cobas (JClinMicro 2012;50:2359-65)

Slide courtesy Dr M Austin, UPMC

Cytology diagnosis and HR-HPV status in biopsy-confirmed HSIL+ lesions

	HR-HPV +	HR-HPV -	TOTAL
HSIL	28	5	33
ASC-H	25	6	31
AGC	7	0	7
CA	1	0	1
LSIL	88	4	92
ASC	62	4	66
NILM	20	3 (1.2%)	23 (9.1%)
Total	231	22 (8.7%)*	253

BioRef/HMH data *Endometrial cancers excluded otherwise 9.4% Published in cancer cytopath 2016

Clinical Performance of the Food and Drug Administration–Approved High-Risk HPV Test for the Detection of High-Grade Cervicovaginal Lesions

Haijun Zhou, MD, PhD[1]; Roxanne R. Mody, MD[2]; Eric Luna, CT (ASCP)[3]; Donna Armylagos, CT (ASCP)[3]; Jiaqiong Xu, PhD[4]; Mary R. Schwartz, MD[1]; Dina R. Mody, MD[1,5]; and Yimin Ge, MD[1,5]

BACKGROUND: In recent years, high-risk human papillomavirus (hrHPV) testing for triaging atypical squamous cells of undetermined significance and cotesting with cytology have been implemented in clinical practice. However, clinical data for primary screening with human papillomavirus (HPV) testing alone are currently lacking. **METHODS:** This study retrospectively reviewed the correlation of cytology, histology, and hrHPV testing through the use of a cytology laboratory quality assurance database with 130,648 Papanicolaou (Pap) tests interpreted at Houston BioReference Laboratories and Houston Methodist Hospital between March 1, 2013 and June 30, 2014. Among the 47,499 patients who had undergone cytology-HPV cotesting, 1654 underwent follow-up biopsies. **RESULTS:** The sensitivities of the hrHPV and Pap tests were 80.8% and 81.2%, respectively, for detecting any type of cervicovaginal dysplasia and 91.3% and 90.9%, respectively, for high-grade cervicovaginal lesions. For biopsy-confirmed high-grade cervicovaginal lesions (cervical intraepithelial neoplasia grade 2+, adenocarcinoma in situ, or carcinoma; n = 253), the false-negative rates for hrHPV and Pap tests were 8.7% and 9.1%, respectively. The false-negative rate for cytology-hrHPV cotesting was only 1.2%. **CONCLUSIONS:** In clinical practice, the hrHPV test alone is not significantly superior to the Pap test as a primary screening method for cervicovaginal lesions. The false-negative rate of the hrHPV test in detecting biopsy-confirmed high-grade cervicovaginal lesions is comparable to the rate of the Pap test. Women with cytology and hrHPV cotesting, however, have a significantly lower false-negative rate than those undergoing either test alone. Currently, cytology-HPV cotesting remains the best strategy for detecting high-grade cervicovaginal lesions. ***Cancer Cytopathol* 2016;124:317-23. © 2016 American Cancer Society.**

KEY WORDS: College of American Pathologists (CAP) benchmark; cotesting; high-grade cervicovaginal lesion; human papillomavirus (HPV) test; Papanicolaou (Pap) test.

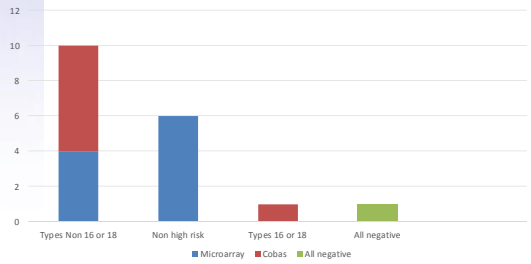
INTRODUCTION

Persistent infections with high-risk human papillomavirus (hrHPV) cause most cervical cancers and precancerous lesions.[1] In the past 50 years, the incidence and mortality of cervical cancer have significantly decreased in the

Cancer cytopathology vol 124(5) 317-23 2016

Follow up to our original paper

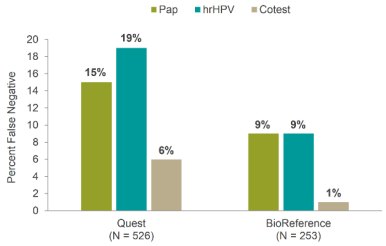
Follow up HPV results on 21 cases where blocks were available



Paper in print in JASC

Comparison of Two Recent Real World Publications From the USA

Figure 1. False Negative Rates by Test Method from Two Real World Studies



Mody D R comparison in lay press (clpmag.com June 2016)
Quest data(Blatt et al) based largely on conventional smears and HCII platform, Bio data(Zhou H et al) on imaged liquid based and Cobas platform

Cervical Squamous Cell carcinoma and Preceding HPV Negative Vs Cytology

Reference	Number	%HPV Neg	% Cytology Neg
Farnsworth A* Acta Cytol 2011;55:307-12	26	8	14
Zaibo I** Arch Pathol and lab med 2015 PMID 24694342	31	10	0
Katki* HA. Lancet oncol. 2011;12(7)663-672	49	37	31
Miller R et al (HMH+SD)(Archives) PMID 26030246	14	21	14
Quest Diagnostics Blatt A et al Cancer Cytopath PMID 25864682	309	19	15
Zheng B 2015(China) CC PMID 2595482	364	5	1.5

•Conventional smears
•** ThinPrep imaged with HCII testing

Cervical Adenocarcinoma and Preceding HPV Negative Vs Cytology Negative

Reference	Number	%HPV neg	% cytology neg
Farnsworth A Acta Cytol 2011;55:307-12	5	80	40
Zaibo I APLM 2015 PMID 24694342	n/a	n/a	n/a
Katki HA. Lancet oncol. 2011;12(7)663-672	27	22	23
Quest Blatt A et al CancerCytopath PMID 25864682	169	26.6	20.7
Zheng 2015 CancerCytopath PMID 2595482	42	25	5.6
Conrad et al 2018 Cancer Cytopath PMID 30351473	45	22.2	4.4 neg, 2.2 unsat, 4.4 ASC-US

Causes of False Negative HPV Results

- No specimen in vial
- Different HPV type than pool in detection assay or non HPV related cancer
- Low viral copy number of HPV
- Presence of inhibitors
- Limitation of analytic sensitivity
- Inadequate specimen

HPV Negative Cervical Cancers vs False Negative HPV Results

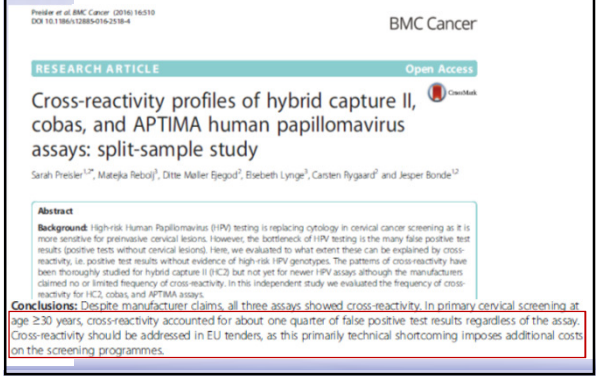
- HPV not detected in 9.4% of cervical cancers
- Additional 3.2% had rare HPV subtypes which are not tested for currently
- 90% of endocervical adenocarcinomas are HPV positive. i.e 10% are negative

Hopenhayn et al *J Low Genit Tract Dis* 2014; 18:182-189
Zhao et al. *Arch Pathol Lab Med* 2014

What About False positives?

- Cross reactivity with low risk types
- Carry over issues..0.35% on Aptima and 0.71% on Cobas
- Other infections
- Lubricants

Cross Reactivity Issues



Preisler et al. BMC Cancer (2016) 16:510
DOI 10.1186/s12885-016-2518-4

BMC Cancer

RESEARCH ARTICLE Open Access

Cross-reactivity profiles of hybrid capture II, cobas, and APTIMA human papillomavirus assays: split-sample study

Sarah Preisler[1,2*], Matejka Rebolj[3], Ditte Møller Bjerrod[2], Elsebeth Lynge[3], Carsten Rygaard[2] and Jesper Bonde[1,2]

Abstract

Background: High-risk Human Papillomavirus (HPV) testing is replacing cytology in cervical cancer screening as it is more sensitive for preinvasive cervical lesions. However, the bottleneck of HPV testing is the many false positive test results (positive tests without cervical lesions). Here, we evaluated to what extent these can be explained by cross-reactivity, i.e. positive test results without evidence of high-risk HPV genotypes. The patterns of cross-reactivity have been thoroughly studied for hybrid capture II (HC2) but not yet for newer HPV assays although the manufacturers claimed no or limited frequency of cross-reactivity. In this independent study we evaluated the frequency of cross-reactivity for HC2, cobas, and APTIMA assays.

Conclusions: Despite manufacturer claims, all three assays showed cross-reactivity. In primary cervical screening at age ≥30 years, cross-reactivity accounted for about one quarter of false positive test results regardless of the assay. Cross-reactivity should be addressed in EU tenders, as this primarily technical shortcoming imposes additional costs on the screening programmes.

F. Test results may be affected by improper specimen collection, storage, or specimen processing.
G. The Internal Control monitors the target capture, amplification, and detection steps of the assay. It is not intended to control for cervical sampling adequacy.
H. A negative APTIMA HPV 16 18/45 Genotype Assay result does not exclude the possibility of cytologic abnormalities or of future or underlying CIN2, CIN3, or cancer.
I. The APTIMA HPV 16 18/45 Genotype Assay provides qualitative results. Therefore, a correlation cannot be drawn between the magnitude of a positive assay signal and the expression level of mRNA in a specimen.
J. Detection of high-risk HPV (types 16, 18, and 45) mRNA is dependent on the number of copies present in the specimen and may be affected by specimen collection methods, patient factors, stage of infection and the presence of interfering substances.
K. Infection with HPV is not an indicator of cytologic HSIL or underlying high-grade CIN, nor does it imply that CIN2, CIN3, or cancer will develop. Most women infected with one or more high-risk HPV types do not develop CIN2, CIN3, or cancer.
L. The following may interfere with the performance of the assay when present at concentrations greater than those specified: vaginal lubricants (containing Polyquaternium 15) at 1% w/v, anti- fungal cream (containing tioconazole) at 0.03% w/v, mucus at 0.3% w/v, vaginal hormones (containing progesterone) at 1% w/v, Trichomonas vaginalis at 3 x 10^4 cells/mL.
M. The effects of other potential variables such as vaginal discharge, use of tampons, douching, etc. and specimen collection variables have not been evaluated.
N. Use of this device must be limited to personnel trained in the use of the APTIMA HPV 16 18/45 Genotype Assay.
O. Cross-contamination of samples can cause false positive results. The carryover rate of the APTIMA HPV 16 18/45 Genotype Assay on the TIGRIS DTS System has been determined in a non-clinical study to be 0.35%.
P. The APTIMA HPV 16 18/45 Genotype Assay should be interpreted in conjunction with other laboratory and clinical data available to the clinician.

Package Insert Cobas, page 14

4. Use of this product must be limited to personnel trained in the techniques of PCR and the use of the **cobas**® 4800 System.
5. The **cobas**® 4800 System includes the **cobas x** 480 instrument and **cobas z** 480 analyzer together with the control unit. This is the only configura product. No other sample preparation instrument or PCR system can be used with this product.
6. Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform met qualify technology differences.
7. The effects of other potential variables such as vaginal discharge, use of tampons, douching, etc. and specimen collection variables have not been
8. Though rare, mutations within the highly conserved regions of the genomic DNA of Human papillomavirus covered by the **cobas**® HPV Test's pr detect the presence of the viral DNA.
9. The presence of PCR inhibitors may cause false negative or invalid results.
10. Cervical specimens often show visibly detectable levels of whole blood as a pink or light brown coloration. These specimens are processe concentrations of whole blood exceed 1.5% (dark red or brown coloration) in PreservCyt® Solution or 2% in SurePath™ Preservative Fluid prior to there is a likelihood of obtaining a false-negative result. The **cobas**® HPV Test performance has not been validated with specimens which ha removal of red blood cells. Any such processing of specimens prior to HPV testing would invalidate the **cobas**® HPV Test results.
11. Cross-contamination of samples can cause false positive results. The sample to sample cross-contamination rate of the **cobas**® HPV Test on the in a non-clinical study to be 0.71% when alternating very high positive and negative samples were tested over multiple runs using both Prese Fluid. The cross-contamination study using SurePath™ Preservative Fluid also produced 11 negative samples with signal above the clinical cu of these results remained negative. In an analytical study using post-cytology PreservCyt® primary vials, the percent of negative clinical spe (95% CI: 4.7% to 29.8%) when processed subsequent to moderate to high positive PreservCyt® clinical specimens on the ThinPrep T3000 processo cutoff of the assay and the results remained negative.

No cross-contamination was seen on the T2000 processor with PreservCyt® specimens. A 1.4% cross-contamination rate was noted with SurePath

5641268001-13EN 14

Cobas vs Aptima (DNA vs RNA)

Performance of Aptima and Cobas HPV Testing Platforms in Detecting High-Grade Cervical Dysplasia and Cancer

Yimin Ge, MD[1,2]; Paul Christensen, MD[1]; Eric Luna, CT (ASCP)[3]; Donna Armylagos, CT (ASCP)[3]; Mary R. Schwartz, MD[1]; and Dina R. Mody, MD[1,2]

BACKGROUND: Human papillomavirus (HPV) tests and genotyping have been used in clinical risk assessment. The purpose of this study was to analyze the performance of 2 common HPV testing platforms in detecting high-grade cervical lesions (high-grade squamous intraepithelial lesion (HSIL) or worse [≥HSIL]). **METHODS:** Between January 1 and December 31, 2015, 2041 Papanicolaou (Pap) tests with biopsy confirmation were analyzed along with HPV tests performed on Cobas or Aptima platforms. A biopsy diagnosis of grade 2 cervical intraepithelial neoplasia was confirmed with p16/Ki-67 immunohistochemistry. **RESULTS:** In total, 1866 and 175 Pap cases were tested on Cobas and Aptima platforms, respectively. Both platforms were highly sensitive (97% for both) for biopsy-confirmed ≥HSIL. Cobas HPV testing had higher positive rates for the diagnosis of benign lesions (84% vs 51%) and low-grade squamous intraepithelial lesions (89% vs 63%) on biopsy compared with Aptima. Aptima testing had significantly higher specificity for ≥HSIL than Cobas (41% vs 13%; $P < .0001$). Overall, performance of the Aptima platform was superior to that of the Cobas platform in detecting biopsy-confirmed ≥HSIL, resulting from its significantly higher positive predictive value (25% vs 16%; $P < .03$) and overall accuracy (50% vs 26%; $P < .0001$). **CONCLUSIONS:** Although both the Cobas and Aptima platforms offer highly sensitive tests for high-grade cervical lesions, Aptima HPV testing demonstrated significantly higher specificity and positive predictive value than Cobas testing for biopsy-confirmed ≥HSIL. The considerable difference may be related to the significant increase in E6/E7 expression after HPV DNA integration. The significantly higher specificity and overall accuracy of Aptima testing for ≥HSIL, resulting in the identification of high-risk populations that require immediate treatment and close follow-up, may prove useful in clinical risk stratification. *Cancer Cytopathol* 2017;000:000-000. © 2017 American Cancer Society.

KEY WORDS: Aptima human papillomavirus (HPV) test; cervical cancer; Cobas HPV test; high-grade squamous intraepithelial lesion (HSIL); HPV E6/E7 messenger RNA test; Papanicolaou (Pap) test.

2017
PMID:
28574670

Cobas vs Aptima (DNA vs RNA)

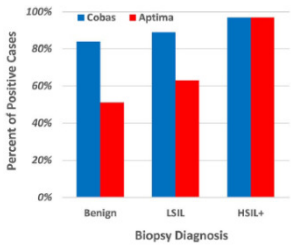


Figure 1. A comparison of results from the Cobas and Aptima human papillomavirus (HPV) testing platforms is illustrated based on HPV-positive rates in cervical biopsy categories.

Cobas vs Aptima (DNA vs RNA)

Variable	Cobas	Aptima	P value
Sensitivity	97	97	.55
Specificity	13	41	<.0001
PPV	16	25	.03
NPV	97	98	1.0
Accuracy	26	50	<.0001

TABLE 2. Performance of Cobas and Aptima Human Papillomavirus Testing in Detecting Biopsy-Confirmed High-Grade Cervical Lesions

	Cobas		Aptima		
Variable	Percentage (No./Total No.)	95% CI, %	Percentage (No./Total No.)	95% CI, %	*P*
Sensitivity	97 (271/278)	95-99	97 (28/29)	80-100	.55
Specificity	13 (209/1588)	12-15	41 (60/146)	33-50	< .0001
PPV	16 (271/1650)	15-18	25 (28/114)	17-34	.03
NPV	97 (209/215)	93-99	98 (60/61)	90-100	1.0
Overall accuracy	26 (480/1866)	24-28	50 (88/175)	43-58	< .0001

Abbreviations: CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.

Cancer Cytopathology Month 2017 PMID:28574670 3

HPV Reporting Rates, CAP Survey

Categories	N	Mean	10th	25th	50th	75th	90th
Total HPV tested volume reported positive	463	22.60	10.0	13.8	20.0	28.4	38.5
ASC-US in women 30 years of age or older (CHPV)	43	31.91	19.8	25.0	30.7	38.3	53.3
ASC-US in women younger than 30 years of age (CHPV)	32	47.73	25.9	42.1	51.3	56.5	63.2
ASC-US (PAP)	110	37.05	11.8	26.4	38.3	47.8	54.7
ASC-H	103	39.87	0.0	1.0	53.8	68.1	79.0
NILM Pap test co-test in women over 30 years of age	81	10.91	2.1	4.4	6.5	11.0	22.5
AGC	90	16.47	0.0	0.0	13.2	27.0	39.3
LSIL in postmenopausal women	41	31.15	0.0	0.8	20.0	64.1	76.8

Zhao C et al Archives of Pathology and Laboratory Medicine 2015 PMID 25436905
Table 5. High-risk HPV Positive Rate Percentage
ASC-US indicates atypical squamous cells of undetermined significance; ASC-H, atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion; LSIL, low-grade intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; AGC, atypical glandular

HPV testing in USA (Cervicovaginal)

- 2001 ASC-US triage (HCII first to get FDA approval, others followed)
- 2003 Co-testing in women 30 and above(HCII first to get FDA approval, others followed)
- 2014 Primary screening (Cobas first one with FDA approval for primary screening and triage and co testing)
- 2018 Primary screening with BD Viper (and triage and co testing)

Evolving Role of HPV testing in Cervicovaginal cytology

- Triage
  - ASC-US
  - LSIL
  - Secondary triage
- Co-testing
  - Women ≥ 30 yrs
  - Test of Cure
- Risk Stratification and management guidelines
- Primary Screening
- Quality Improvement

HPV Reporting Rates in Different Trials

Trial/Survey	Assay	NILM	ASC-US	ASC-H
CAP 50th	Various&HCII	6.5%(>30)	38.3	50
ALTS(USA)	HCII	32.7%	50.6%	75.9%
ATHENA(USA	Cobas	9.8%(>25)	31.8%	
CLEAR(USA)	Aptima	5%(>30yrs)	41.8%	
CCCaST(Can)	HCII	5.2%	31%	
ARTISTIC(UK)	HCII	10.4%	31.1%	
POBASCAM(Netherlands)	PCR	3.6%	27.4%	
Sweedscreen	PCR	5.5%	63.6%	

Chapter on HPV testing by M.J Thrall in Diagnostic Pathology: Cytopathology by Mody, 2014 and 2018

ALTS Trial and HPV testing

- **LSIL & ASC-H: >80% HPV+; not effective triage**
- **HPV+ ASC-US and LSIL both have 26-28% cumulative risk of CIN2+ at 2 years; can be followed similarly after colposcopy (12 month HPV OR 6 and 12 month Paps)**
- **HC-2 viral load not predictive of severity of disease**

Role of HPV testing in Cervicovaginal cytology

- Risk Stratification and Management
- Triage
  - ASC-US
  - LSIL
  - Secondary triage
- Co-testing
  - Women ≥ 30 yrs
  - Test of Cure
- Primary Screening
- Quality Improvement

HPV and Pap co-testing

- Beginning at age 30
- If double negative, then extended screening interval (5yrs in USA)(2012 guidelines)*
- If Pap negative and HPV positive, then repeat in 1 yr. If still + then colpo. If pap at ASC-US or > then colpo
- Or Genotype. If 16 or 18+ then immediate colposcopy. Othe12 types, repeat in 1 yr
- Not recommended for women <30 due to increased prevalence

High Risk HPV Testing Table

Age	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
Post-Menopausal	1.1#	1.2	1.3	2.5	2.5	2.5

Cell color indicates if HPV testing is appropriate (green) or not appropriate (red).
Numbers in table cells refer to text outline.

#For women 30 and older who are both cytology and HPV negative, repeat both tests only after a 3-year interval.

*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 post-colposcopy management.

Commentary Published by CETC simultaneously In multiple cytology journals PMID:25228434

AGC and HPV Testing Notes…

- HPV testing not the primary management algorithm (see ASCCP management guidelines at www.ASCCP.org)
- HPV positivity points towards Cervical preneoplastic/neoplastic condition
- HPV negativity does not rule out endometrial or extrauterine malignancy or pathology

Risk Stratification…5yr Risk of HSIL+ and Suggested Management Based on Test Results

Table 2. Ranked 5-Year Risk of HSIL and Cancer and Suggested Management According to Test Results.*

Result on Cytologic Testing or Cotesting	Frequency of Screening Result†	Risk of Histologic HSIL and Cancer‡ (percent)	Suggested Management
SCC	0.0048	84	Immediate colposcopy
HPV+/HSIL	0.20	71	Immediate colposcopy
HSIL	0.21	69	Immediate colposcopy
HPV-/HSIL	0.013	49	Immediate colposcopy
HPV+/AGC	0.054	45	Immediate colposcopy
HPV+/ASC-H	0.12	45	Immediate colposcopy
ASC-H	0.17	35	Immediate colposcopy
HPV+/LSIL	0.81	19	Immediate colposcopy
HPV+/ASC-US	1.1	18	Immediate colposcopy
LSIL	0.97	16	Immediate colposcopy
AGC	0.21	13	Immediate colposcopy
HPV-/ASC-H	0.051	12	Immediate colposcopy
HPV+/Pap-	3.6	10	Repeat testing in 6 to 12 mo
ASC-US	2.8	6.9	Repeat testing in 6 to 12 mo
HPV-/LSIL	0.19	5.1	Repeat testing in 6 to 12 mo
HPV-/AGC§	0.16	2.2	Immediate colposcopy
HPV-/ASC-US	1.8	1.1	Repeat testing in 3 yr
Pap-	96	0.68	Repeat testing in 3 yr
HPV-/Pap-	92	0.27	Repeat testing in 5 yr

* The data are based on cytologic testing and cotesting performed by Kaiser Permanente Northern California. Under the principle of similar management of similar risks, the management guidelines for cotesting results were "benchmarked" to the current management of cytologic-testing-only results. In accordance with the Bethesda System, AGC denotes

Schiffman M and Solomon D. N Engl J med 369:24 Dec 12, 2013

Risk Stratification…5yr Risk of HSIL+ and Suggested Management Based on Test Results

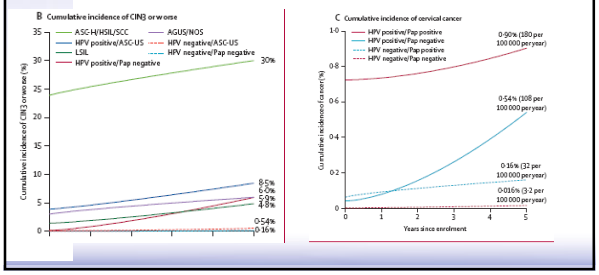
Screen Result	Reporting Frequency %	5 yr Risk of HSIL+	Suggested Management
HPV-/Pap -	92	0.27	Repeat screen in 5 yrs
HPV+/Pap-	3.6	10	Repeat in 6-12 mos
Pap -	96	0.68	Repeat screen in 3 yrs
ASC-US HPV-	1.8	1.1	Repeat screen in 3 yrs
ASC-US HPV+	1.1	18	Immediate Colposcopy
ASC-US	2.8	6.9	Repeat in 6-12 months
LSIL	0.97	16	Immediate Colposcopy
LSIL HPV-	0.19	5.1	Repeat in 6-12 months
LSIL HPV +	0.81	19	Immediate Colposcopy
ASC-H	0.17	35	Immediate Colposcopy
ASC-H HPV+	0.12	45	Immediate Colposcopy
ASC-H HPV -	0.051	12	Immediate Colposcopy
HSIL,AGC,CA,AIS			Immediate Colposcopy

Schiffman M and Solomon D. N Engl J med 369:24 Dec 12, 2013

Cervical cancer risk for women undergoing concurrent testing for human papillomavirus and cervical cytology: a population-based study in routine clinical practice

Hormuzd A Katki, Walter K Kinney, Barbara Fetterman, Thomas Lorey, Nancy E Poitras, Li Cheung, Franklin Demuth, Mark Schiffman, Sholom Wacholder, Philip E Castle

www.thelancet.com/oncology Published online June 17, 2011 DOI:10.1016/S1470-2045(11)70145-0
2011;12(7)663-672



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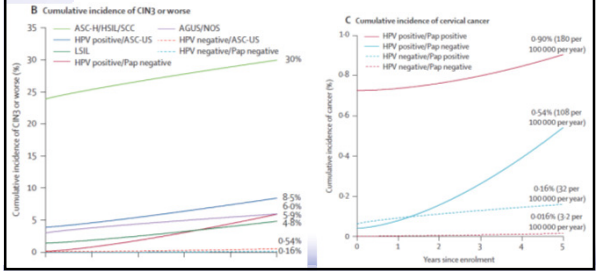


Primary HPV Screening Summary

(Roche Cobas Approved in USA April 2014)

- Roche Cobas detects 14 High risk types
- Types 16, 18 individually identified and the other 12 non 16/18 as a group
- Ages 25 and above
- Internal control of β-globin gene is not cervical epithelial cell specific
- There is limited data re **interfering** substances
- Labs offering primary screening should be enrolled in PT and perform appropriate QC measures (ATHENA trial used ThinPrep)

Nayar, Goulart, Wasserman, Davey Cancer Cytopathology 2014

Primary HPV Screening Suggested Management Algorithm

- If primary screen is 16 or 18 + then refer to colposcopy
- If non 16/18 positive, then reflex to cytology
  - If cytology ASC-US or above then manage per cytology
  - Remember, HPV negative squamous and adenocarcinomas of the cervix do occur

Nayar, Goulart, Wasserman, Davey Cancer Cytopathology 2014

Interim Guidance: HPV as Primary Screen one of 3 options (Pap, Pap+HPV, HPV)

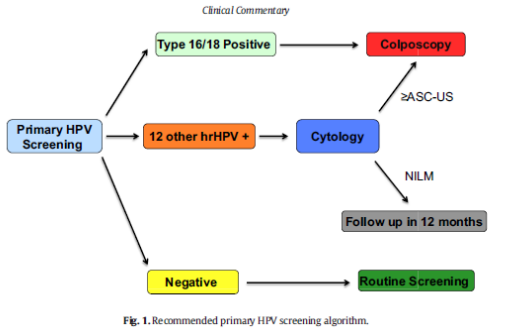


Fig. 1. Recommended primary HPV screening algorithm.

Please cite this article as: Huh WK, et al, Use of primary high-risk human papillomavirus testing for cervical cancer screening: Interim clinical guidance, Gynecol Oncol (2015), http://dx.doi.org/10.1016/j.ygyno.2014.12.022

Issues with Athena trial

- About 33% of women identified with high grade SILs were between 25-29 yrs which would result in an increase in colposcopies
- Only 3 year follow-up data available (at that time), hence best long term screening strategy difficult to determine at this point
- Persistent HPV 16/18+ but colposcopy negative…limited data re appropriate management

Screening Programs

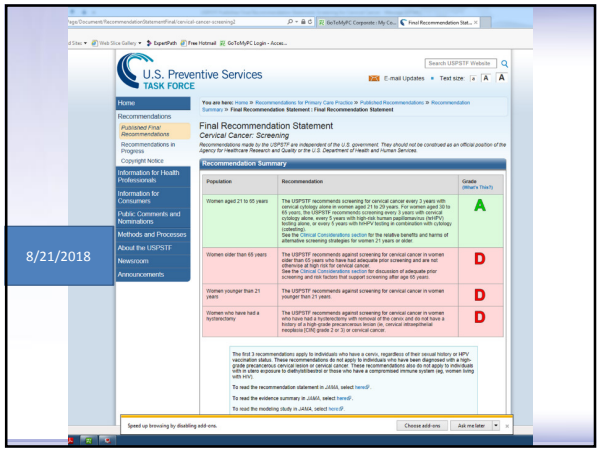
- USA has an opportunistic screening program
- No call and re call mechanisms which automatically generate "invitation" letters
- Most European countries with government paid programs have detailed follow-up set ups with incentives for the provider and automated reminders for the women

Screening Programs..continued

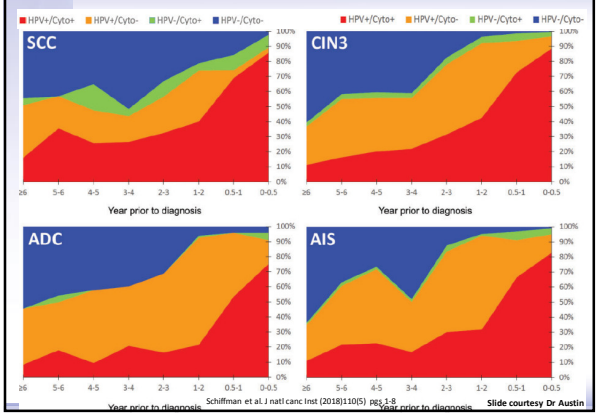
- Extended screening intervals in the US opportunistic system with a large immigrant population could have untoward consequences
- After correction for Hysterectomy, there appears to be a second peak of cervical cancer in the 6th decade in the USA

United States Preventative Services Task Force (USPSTF) draft for Cervical Cancer Screening Guidelines (2018)

- Cytology every 3 yrs in women between 21-29 years
- Every 3 years with cytology alone or every 5 years with HPV alone or PAP+ HPV co testing between 30-65 years
- No screening under 21 or over 65 (for those with adequate screening history and usual risk)
- No screening if hysterectomy (no cervix) for benign disease



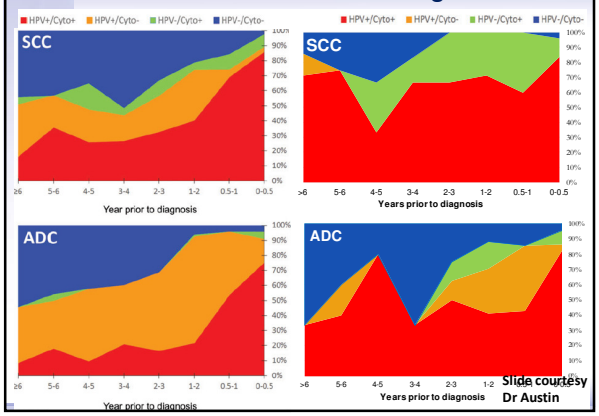
Kaiser Permanente data (JNCI 2018: 110: djx225)



Schiffman et al. J natl canc Inst (2018)110(5) pgs 1-8

Slide courtesy Dr Austin

Cervical Cancer: Kaiser vs. Magee



Slide courtesy Dr Austin

QA of HPV testing in the Implementation of HPV Primary screening in Norway: an inter-laboratory reproducibility study

	NILM N=355	ASC-US N=36	LSIL N=33	ASC-H N=11	HSIL N=38	UNSAT N=27
100 selected samples	0	28	28	11	33	0
399 consecutive samples	354	8	5	0	5	27

5 out of 38 HSILs were negative in all 4 labs=10.5% false negative rate

Engesaeter B et al BMC Infec Dis 2016;16:698 PMCID:PMC5122146

JASC 2018 Jan pgs 50-55

Available online at www.sciencedirect.com
ScienceDirect
journal homepage: www.jascyto.org/
ELSEVIER

SHORT COMMUNICATION

Primary HPV cervical cancer screening in the United States: Are we ready?

Ritu Nayar, MD[a,*], Robert A. Goulart, MD[b], Diane D. Davey, MD[c]

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[b] Cytopathology Services, New England Pathology Associates at Trinity Health of New England, Mercy Medical Center, Springfield, Massachusetts
[c] Department of Clinical Sciences, University of Central Florida, Orlando, Florida

KEYWORDS
cervical cancer;
primary HPV screening;
USPSTF guidelines

In September 2017, the United States Preventive Services Task Force put forth updated draft guidelines for cervical cancer screening in the United States, which were then open to public comment. The recommendations allowed for every-3-year cervical cytology screening in women aged 21 to 65 years with an option for every-5-year high-risk human papillomavirus testing in women aged 30 to 65 years. There was no option

Colposcopy rates

Journal of the American Society of Cytopathology (2017) 6, 180–184
Available online at www.sciencedirect.com
ScienceDirect
journal homepage: www.jascyto.org/
ELSEVIER

ORIGINAL ARTICLE

Cervical biopsy rates before and after the introduction of human papillomavirus type reporting in co-tests with negative cytology

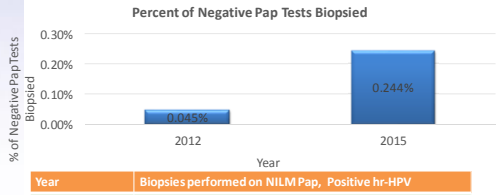
Heather Ruff, DO[a], Dina Mody, MD[a], Eric Luna, MBA, CT(ASCP)[b], Donna Armylagos, BA, CT(ASCP)[b], Michael J. Thrall, MD[a,*]

[a] Department of Pathology and Genomic Medicine, Houston Methodist Hospital, Houston, Texas
[b] BioReference Laboratories, Houston, Texas

Received 12 May 2017; received in revised form 12 June 2017; accepted 13 June 2017

Sept-October 2017 issue of JASCyto…Open access

Biopsies of Negative Pap Tests Increase

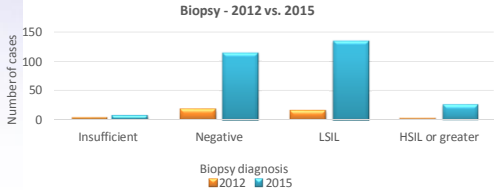


Year	Biopsies performed on NILM Pap, Positive hr-HPV
2012	27 / 82,721 (0.045%)
2015	281 / 115,104 (0.244%)

* P value <.001

Journal of the American Society of Cytopathology (2017) 6, 180-184

Increase Seen In All Diagnosis Categories



Journal of the American Society of Cytopathology (2017) 6, 180-184

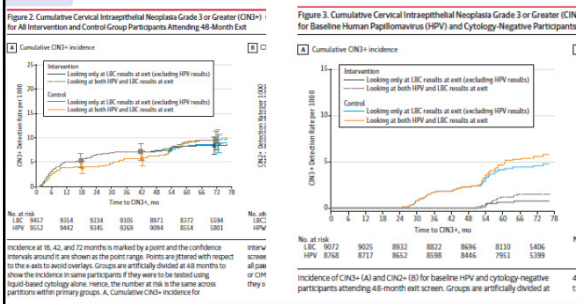
Increase in HSIL Not Statistically Significant

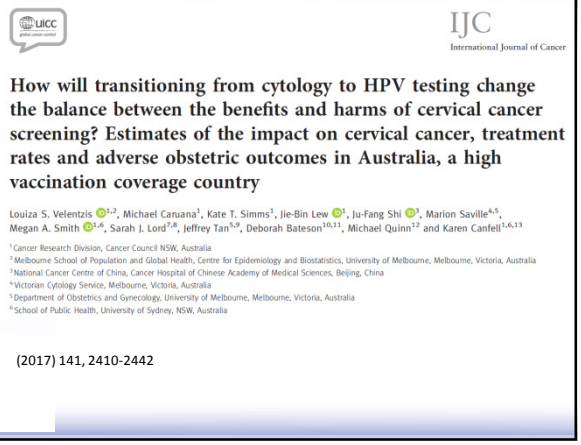
Year	HSIL or greater
2012	2/37 (5.4%)
2015	25/281 (8.9%)

* P value .801

Journal of the American Society of Cytopathology (2017) 6, 180-184

Screening With HPV testing vs Cytology for Cervical Intraepithelial Neoplasia
The HPV FOCAL Randomized Clinical Trial (Canada)
Ogilvie G,Niekerl D, Kradjen M et al in JAMA Vol 320 (1) 43-51. July 3, 2018





How will transitioning from cytology to HPV testing change the balance between the benefits and harms of cervical cancer screening? Estimates of the impact on cervical cancer, treatment rates and adverse obstetric outcomes in Australia, a high vaccination coverage country

Louiza S. Velentzis[1,2], Michael Caruana[1], Kate T. Simms[1], Jie-Bin Lew[1], Ju-Fang Shi[3], Marion Saville[4,5], Megan A. Smith[1,6], Sarah J. Lord[7,8], Jeffrey Tan[5,9], Deborah Bateson[10,11], Michael Quinn[12] and Karen Canfell[1,6,13]

[1] Cancer Research Division, Cancer Council NSW, Australia
[2] Melbourne School of Population and Global Health, Centre for Epidemiology and Biostatistics, University of Melbourne, Melbourne, Victoria, Australia
[3] National Cancer Centre of China, Cancer Hospital of Chinese Academy of Medical Sciences, Beijing, China
[4] Victorian Cytology Service, Melbourne, Victoria, Australia
[5] Department of Obstetrics and Gynecology, University of Melbourne, Melbourne, Victoria, Australia
[6] School of Public Health, University of Sydney, NSW, Australia

(2017) 141, 2410-2442

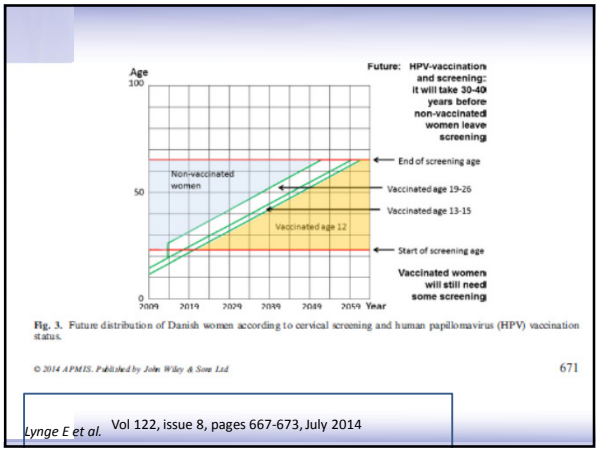


Fig. 3. Future distribution of Danish women according to cervical screening and human papillomavirus (HPV) vaccination status.

Lynge E et al. Vol 122, issue 8, pages 667-673, July 2014

Cytology vs HPV to Screen for Cervical Cancer

Cytology

- Requires screening Infrastructure (CT, schools)
- Cost …depends, but generally lower
- Issues with false negatives and positives
- Colposcopy rates…depends on triage
- Self collected specimens, inferior
- Screening for pre cancer and cancer

HPV

- None required
- Depends on vendor and price, generally more
- Same! (people are just not aware)
- Increased. Can be high depending upon prevalence
- Self collected specimens can be equivocal (if highly sensitive test used)
- Screening for infection that causes most cervical cancers

There is no perfect test!

Role of HPV testing in Cervicovaginal cytology

- Risk Stratification and Management
- Triage
- Co-testing
- Primary Screening
- Quality Improvement
  - Monitoring HPV positivity rates for ASC-US by lab as well as by individuals and benchmarking
  - Cytology-Histology correlation

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ASC-US :SIL ratio and HPV rates as QA monitors in lab

- Tworek JA, Jones BA, Raab S, Clary KM, Walsh MK. The value of monitoring human papillomavirus DNA results for Papanicolaou tests diagnosed as atypical squamous cells of undetermined significance: a College of American Pathologists Q-Probes study of 68 institutions. Arch Pathol Lab Med. 2007 Oct;131(10):1525-31. PubMed PMID: 17922588.
- Cibas ES, Zou KH, Crum CP, Kuo F. Using the rate of positive high-risk HPV test results for ASC-US together with the ASC-US/SIL ratio in evaluating the performance of cytopathologists. Am J Clin Pathol. 2008 Jan;129(1):97-101. PubMed PMID: 18089494.
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- Chebib I, Rao RA, Wilbur DC, Tambouret RH. Using the ASC:SIL ratio, human papillomavirus, and interobserver variability to assess and monitor cytopathology fellow training performance. Cancer Cytopathol. 2013 Nov;121(11):638-43. doi: 10.1002/cncy.21328. Epub 2013 Jul 16. PubMed PMID: 23861336.
- Booth CN, Bashleben C, Filomena CA, Means MM, Wasserman PG, Souers RJ, Henry MR. Monitoring and ordering practices for human papillomavirus in cervical cytology: findings from the College of American Pathologists Gynecologic Cytopathology Quality Consensus Conference working group 5. Arch Pathol Lab Med. 2013 Feb;137(2):214-9. doi: 10.5858/arpa.2012-0114-CP. Review. PubMed PMID: 23368863.

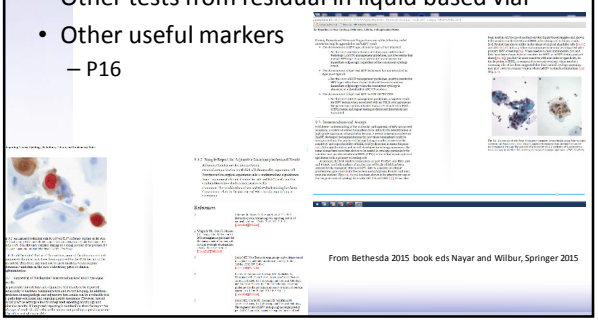
ASC-US to SIL Ratio and HPV positivity rates for ASC-US as QA Indicator

ASC:SIL Ratio	Hr HPV+ASC-US	Interpretation
Normal	Normal	Appropriate
High	Normal	Negs and LSILs being called ASC-US
High	High	LSILs undercalled as ASC-US
High	Low	Normals overcalled as ASC-US
Normal	Low	General overcalling
Normal	High	General undercalling
Low	High	1) Over and undercalling ASC-US 2) ASC-US called LSIL 3) ASC-US called NILM

Adapted from Cibas et al. PMID 8089494…Please refer to article for other scenarios

Other Ancillary Testing

- Other tests from residual in liquid based vial
- Other useful markers
  - P16



From Bethesda 2015 book eds Nayar and Wilbur, Springer 2015

Synopsis of PALMS

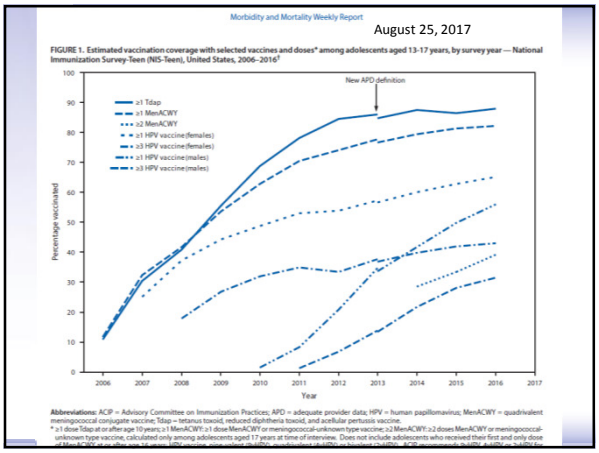
(Primary ASC-US and Low Grade Marker Study)

	CIN2+		CIN3+		CIN2+	
	Sensitivity	Specificity	Sensitivity	Specificity	PPV	NPV
18-65 yrs						
Pap	68.5	95.4	73.6	95.1	13.3	99.7
Dual	86.7	95.2	87.4	94.8	15.6	99.9
18-29 yrs						
Pap	71.9	96.3	69	96.1	14.4	99.5
Dual	89.4	96.2	87.2	95.9	16.1	99.8
30-65 yrs						
Pap	66	96.3	69	96.1	12.5	99.7
Dual	84.7	96.2	87.2	95.9	15.3	99.9
HPV (HCII)	93.3	93	96.2	92.7	9.3	99.9

Pap at ASC-US or worse, Dual=Pap with P16/ki67
Ikenberg et al JNCI Vol 105, issue 20 2013

FDA-approved vaccines

Vaccine Name	Manu-facturer	Types Included	Date of FDA Approval	Efficacy Preventing CIN2-3/AIS
Cervarix	Glaxo-Smith-Kline	16,18	October, 2009	92.9%
Gardasil 4	Merck	16,18 6,11 (low risk)	June, 2006	98.2%
Gardasil 9 (second generation)	Merck	16,18,31,33 45,52,58 6,11 (low risk)	December, 2014	96.3%



HPV Vaccination Review Article


Gynecologic Oncology 146 (2017) 196–204

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Review Article

HPV vaccines – A review of the first decade

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HIGHLIGHTS

- Do not use Gardasil9 as a booster vaccine for those already vaccinated.
- Gardasil9 and Cervarix are equivalent in efficacy against CIN 2 + regardless of HPV type.
- Only two doses of HPV vaccine for 9–15 year olds at 6 month or 1 year intervals.
- Cervarix has 91% efficacy in women older than 25 years lasting for at least 7 years.
- HPV vaccines reduce abnormal screening tests, colposcopies and excisions.

Harper D, Demars L HPV vaccines-A review of the first decade Gynecologic oncology 146 (2017) 196-204

Screening and management Post HPV Vaccination

- Continue with screening as there are other types we are not vaccinating against
- Some "herd" immunity but exact status unknown
- Duration of immunity also unknown….think whooping cough!
- What about colposcopy? Currently the "gold" standard…will it be valid once 16/18 are eliminated

Future Directions


Technology Solutions for Global Health

Rapid Tests for Cervical Cancer

Health need

Technology solution

Current status and results

Availability

Donor support

PATH

Innovation in cervical cancer screening

New tests offer hope for women in the developing world

Meeting the need for appropriate screening tools

"There is sufficient evidence that testing for human papillomavirus infection as the primary screening modality can reduce cervical cancer incidence and mortality rates."

SOCIOLOGY OF HEALTH & ILLNESS

Sociology of Health & Illness Vol. 34 No. 2 2012 ISSN 0141-9889, pp. 234–250
doi: 10.1111/j.1467-9566.2011.01411.x

A molecular monopoly? HPV testing, the Pap smear and the molecularisation of cervical cancer screening in the USA

Stuart Hogarth[1], Michael M. Hopkins[2] and Victor Rodriguez[3]

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Abstract DNA-based molecular testing for human papillomavirus has emerged as a novel approach to cervical cancer screening in the context of well-entrenched existing technology, the Pap smear. This article seeks to elucidate the process of molecularisation in the context of screening programmes. We illustrate how, although Pap has long been problematised and could be seen as a competing technological option, the existing networks and regime for Pap were important in supporting the entrenchment process for the artefacts, techniques and new diagnostics industry entrant, Digene, associated with the new test. The article provides insights into how the molecularisation of screening unfolds in a mainstream market. We reveal an incremental and accretive, rather than revolutionary, process led by new commercial interests in an era when diagnostic innovation is increasingly privatised. We show Digene's reliance on patents, an international scientific network and their position as an obligatory point of

COLLEGE of AMERICAN PATHOLOGISTS
Laboratory Quality Solutions

Human Papillomavirus (HPV) Testing in Head and Neck Carcinomas Guideline

The College of American Pathologists 2017 evidence-based guideline details testing for HPV in certain types of head and neck cancers. Patients with HPV-positive oropharyngeal squamous cell carcinomas (OPSCCs) have a better prognosis and may be candidates for less aggressive treatment.

Pathologists...

SHOULD perform high-risk (HR) HPV on all patients with newly diagnosed OPSCC

SHOULD NOT routinely perform HR-HPV testing on patients with nonoropharyngeal primary tumors of the head and neck

SHOULD NOT provide a tumor grade or differentiation status for HPV-positive/p16-positive OPSCCs

This guideline will help to ensure:

- Eligible patients receive consistent, accurate assessment of HPV status
- Clinicians design and deliver treatment plans tailored to the pathology and needs of each patient

Human Papillomavirus Testing in Head and Neck Carcinomas

Guideline From the College of American Pathologists

James S. Lewis Jr, MD; Beth Beadle, MD, PhD; Justin A. Bishop, MD; Rebecca D. Chernock, MD; Carol Colasacco, MLIS, SCT(ASCP); Christina Lacchetti, MHSc; Joel Todd Moncur, MD, PhD; James W. Rocco, MD, PhD; Mary R. Schwartz, MD; Raja R. Seethala, MD; Nicole E. Thomas, MPH, CT(ASCP)[CM]; William H. Westra, MD; William C. Faquin, MD, PhD

• *Context.*—Human papillomavirus (HPV) is a major cause of oropharyngeal squamous cell carcinomas, and HPV (and/or surrogate marker p16) status has emerged as a prognostic marker that significantly impacts clinical management. There is no current consensus on when to test oropharyngeal squamous cell carcinomas for HPV/p16 or on which tests to choose.

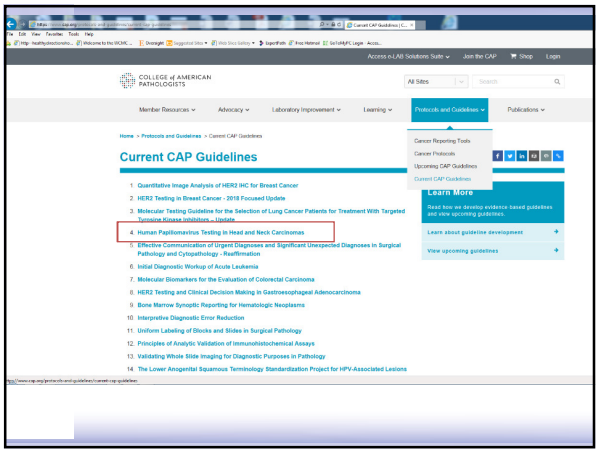
Objective.—To develop evidence-based recommendations for the testing, application, interpretation, and reporting of HPV and surrogate marker tests in head and neck carcinomas.

Design.—The College of American Pathologists convened a panel of experts in head and neck and molecular pathology, as well as surgical, medical, and radiation oncology, to develop recommendations. A systematic review of the literature was conducted to address 6 key questions. Final recommendations were derived from strength of evidence, open comment period feedback, and expert panel consensus.

Results.—The major recommendations include (1) testing newly diagnosed oropharyngeal squamous cell carcinoma patients for high-risk HPV, either from the primary tumor or from cervical nodal metastases, using p16 immunohistochemistry with a 70% nuclear and cytoplasmic staining cutoff, and (2) not routinely testing nonsquamous oropharyngeal carcinomas or nonoropharyngeal carcinomas for HPV. Pathologists are to report tumors as HPV positive or p16 positive. Guidelines are provided for testing cytologic samples and handling of locoregional and distant recurrence specimens.

Conclusions.—Based on the systematic review and on expert panel consensus, high-risk HPV testing is recommended for all new oropharyngeal squamous cell carcinoma patients, but not routinely recommended for other head and neck carcinomas.

(*Arch Pathol Lab Med.* 2018;142:559–597; doi: 10.5858/arpa.2017-0286-CP)



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1. Quantitative Image Analysis of HER2 IHC for Breast Cancer
2. HER2 Testing in Breast Cancer - 2018 Focused Update
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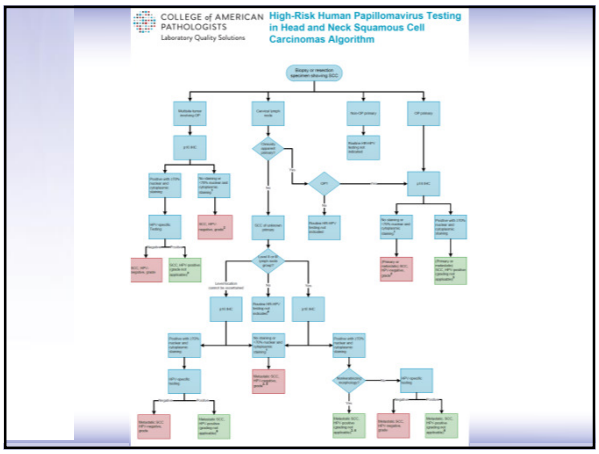
Human Papillomavirus Testing in Head and Neck Carcinomas

Statements and Strength of Recommendations

Summary of Recommendations

	Guideline Statement	Strength of Recommendation
1.	Pathologists should perform high-risk human papillomavirus (HR-HPV) testing on all patients with newly diagnosed oropharyngeal squamous cell carcinoma (OPSCC), including all histologic subtypes. This testing may be performed on the primary tumor or on a regional lymph node metastasis when the clinical findings are consistent with an oropharyngeal primary.	Strong Recommendation
2.	For oropharyngeal tissue specimens (ie, noncytology), pathologists should perform HR-HPV testing by surrogate marker p16 immunohistochemistry (IHC). Additional HPV-specific testing may be done at the discretion of the pathologist and/or treating clinician, or in the context of a clinical trial.	Recommendation
3.	Pathologists should *not* routinely perform HR-HPV testing on patients with nonsquamous carcinomas of the oropharynx.	Expert Consensus Opinion
4.	Pathologists should *not* routinely perform HR-HPV testing on patients with nonoropharyngeal primary tumors of the head and neck.	Recommendation
5.	Pathologists should routinely perform HR-HPV testing on patients with metastatic squamous cell carcinoma (SCC) of unknown primary in a cervical upper or mid jugular chain lymph node. An explanatory note on the significance of a positive HPV result is recommended.	Recommendation
6.	For tissue specimens (ie, noncytology) from patients presenting with metastatic SCC of unknown primary in a cervical upper or mid jugular chain lymph node, pathologists should perform p16 IHC. Note: Additional HR-HPV testing on p16-positive cases should be performed for tumors located outside of level II or III (nonroutine testing) in the neck and/or for tumors with keratinizing morphology.	Expert Consensus Opinion
7.	Pathologists should perform HR-HPV testing on head and neck fine needle aspiration (FNA) SCC samples from all patients with known OPSCC not previously tested for HR-HPV, with suspected OPSCC, or with metastatic SCC of unknown primary. Note: No recommendation is made for or against any specific testing methodology for HR-HPV testing in FNA samples. If the result of HR-HPV testing on the FNA sample is negative, testing should be performed on tissue if it becomes available. If pathologists use cytology samples for p16 IHC testing, they should validate the criteria (ie, cutoff) for a positive result.	Expert Consensus Opinion

8.	Pathologists should report p16 IHC positivity as a surrogate for HR-HPV in tissue specimens (i.e., noncytology) when there is at least 70% nuclear and cytoplasmic expression with at least moderate to strong intensity.	Expert Consensus Opinion
9.	Pathologists should *not* routinely perform low-risk HPV testing on patients with head and neck carcinomas.	Expert Consensus Opinion
10.	Pathologists should *not* repeat HPV testing on patients with locally recurrent, regionally recurrent, or persistent tumor if primary tumor HR-HPV status has already been established. If initial HR-HPV status was never assessed or results are unknown, testing is recommended. HPV testing may be performed on a case-by-case basis for diagnostic purposes if there is uncertainty regarding whether the tumor in question is a recurrence or a new primary SCC.	Expert Consensus Opinion
11.	Pathologists should *not* routinely perform HR-HPV testing on patients with distant metastases if primary tumor HR-HPV status has been established. HPV testing may be performed on a case-by-case basis for diagnostic purposes if there is uncertainty regarding whether the tumor in question is a metastasis or a new primary SCC.	Expert Consensus Opinion
12.	Pathologists should report primary OPSCCs that test positive for HR-HPV or its surrogate marker p16 as HPV-positive and/or p16-positive.	Expert Consensus Opinion
13.	Pathologists should *not* provide a tumor grade or differentiation status for HPV-positive/p16-positive OPSCCs.	Expert Consensus Opinion
14.	Pathologists should *not* alter HR-HPV testing strategy based on patient smoking history.	Expert Consensus Opinion



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- Joel T. Moncur, MD, PhD
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