Role of HPV and Ancillary testing in Gynecology Cytology: From Primary Screen to Management and Quality Assurance

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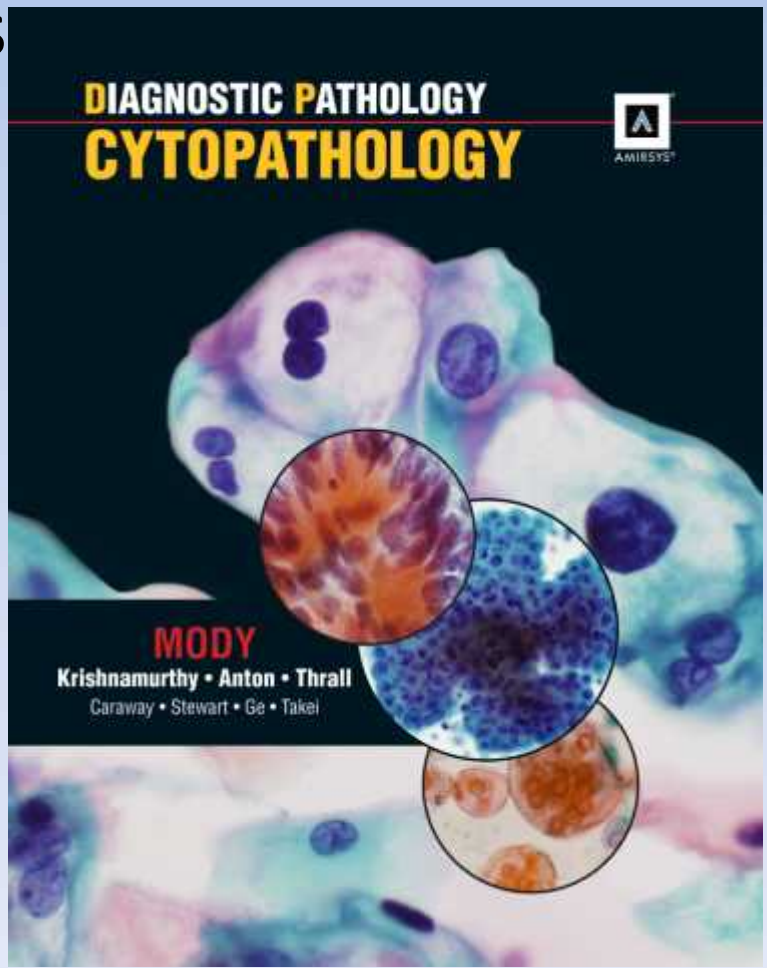
Conflict of Interest

- None with vendors of cytology equipment/testing/vaccines
- Amirsys (now Elsevier)



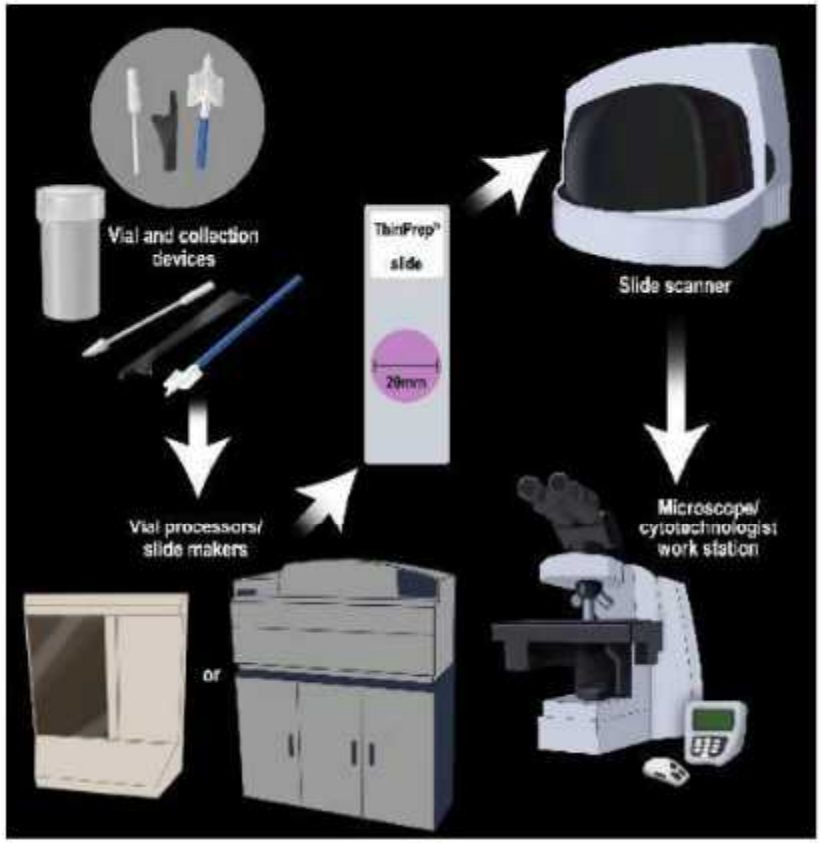
For This Talk……

- A brief historical perspective
- HPV facts and testing types
- Issues with HPV testing
- Current Role of HPV testing
  - HPV testing as triage
  - HPV co-testing
  - HPV as primary screen
  - HPV for QA
  - HPV benchmarking
- Future screening strategies

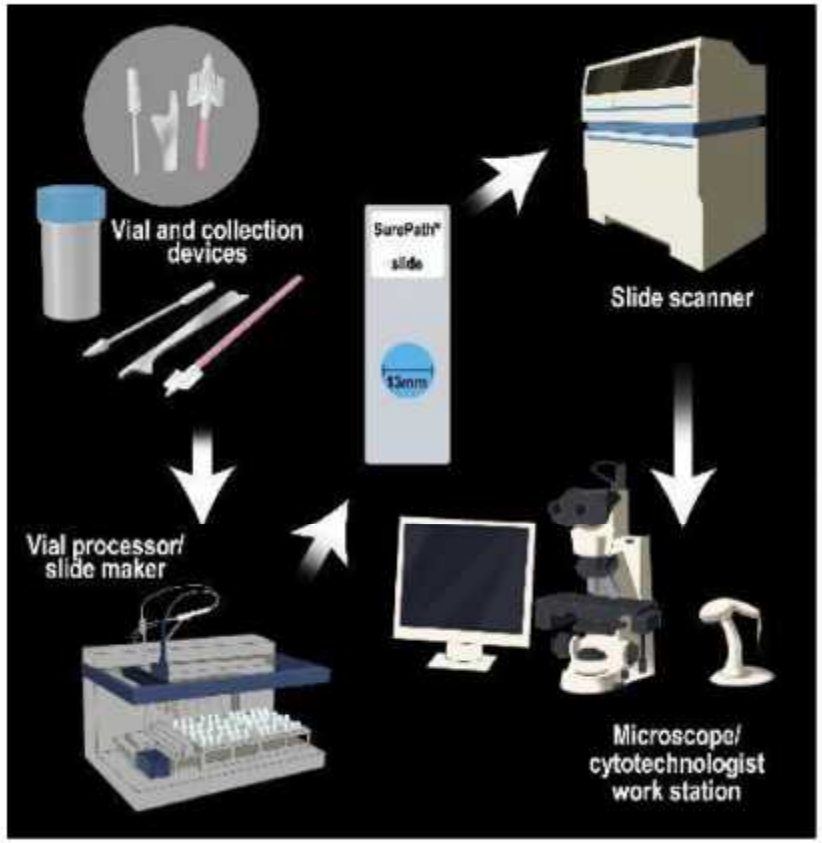
HPV Facts…continued

- 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

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 M J Thrall in Diagnostic Pathology: Cytopathology Mody: Amirsys 2014

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**	Gen Probe	2011	RNA(E7mRNA)	16,18 + 12 types

*No internal control

** Hologic purchased in Aug 2012

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

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
P16 on cytology	85.7	54.7	49.1
Cytology (CIN1 or>)	88.9	58.1	50.7
Hologic	n/a	n/a	n/a

*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

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

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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.

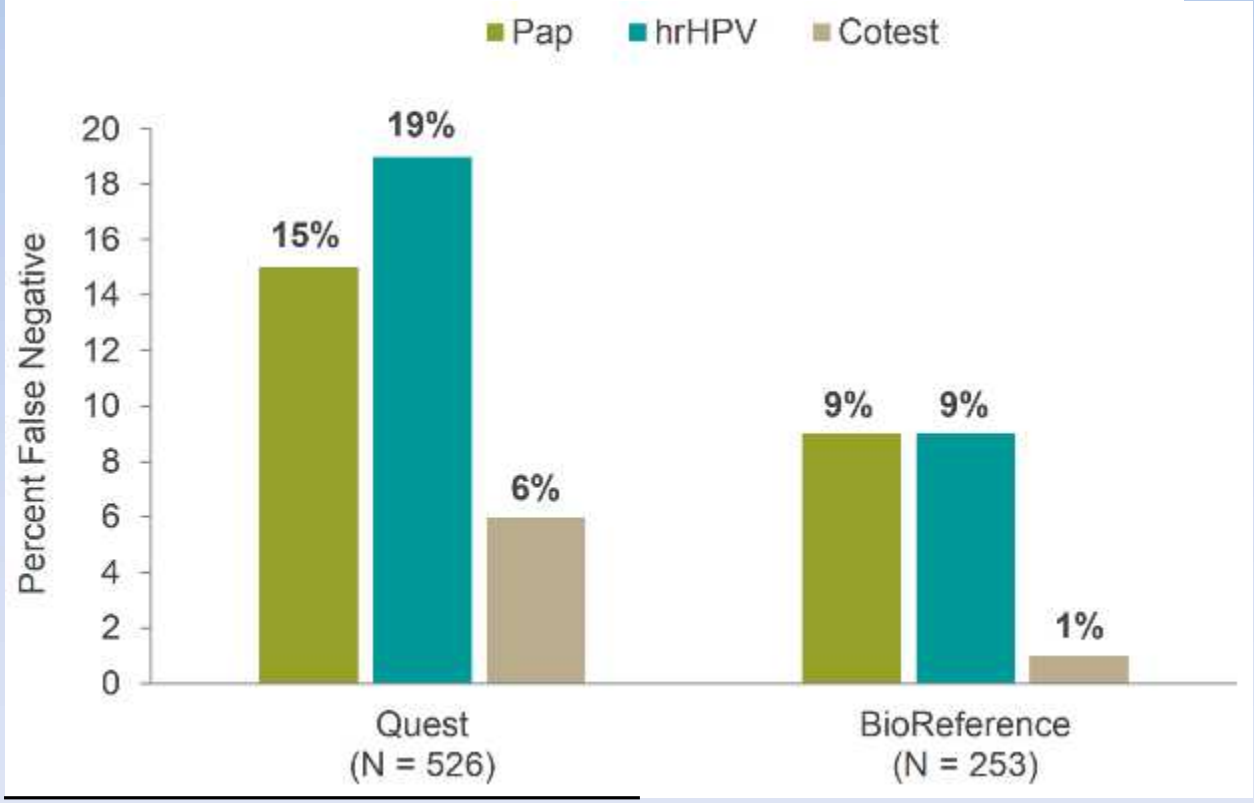
INTRODUCTION

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

Two Recent Real World Publications From the USA

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



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
- 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
  - Interim clinical guidance document by ASCCP/SGO is published. Primary HPV screen is one of 3 options (Pap 3yrs, PAP+HPV 5yrs, HPV 3yrs)
  - Remember, HPV negative squamous and adenocarcinomas of the cervix do occur

Nayar, Goulart, Wasserman, Davey Cancer Cytopathology 2014

Causes of False Negative HPV Results

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

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 3×10^4 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.

Role of HPV testing in Cervicovaginal cytology

- Triage
  - ASC-US
  - LSIL
  - Secondary triage
- Co-testing
  - Women ≥ 30 yrs
  - Test of Cure
- Primary Screening
  - Cobas FDA approved for women≥ 25 yrs (one of 3 options: Pap q3 yrs, co test q 5yrs, HPV q3yrs)

Quality Improvement
Risk Stratification

Role of HPV testing in Cervicovaginal cytology

- Risk Stratification and Management
- Triage
- Co-testing
- Primary Screening
- Quality Improvement

Role of HPV testing in Cervicovaginal cytology

- Risk Stratification and Management
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Role of HPV testing in Cervicovaginal cytology

- Triage
  - ASC-US
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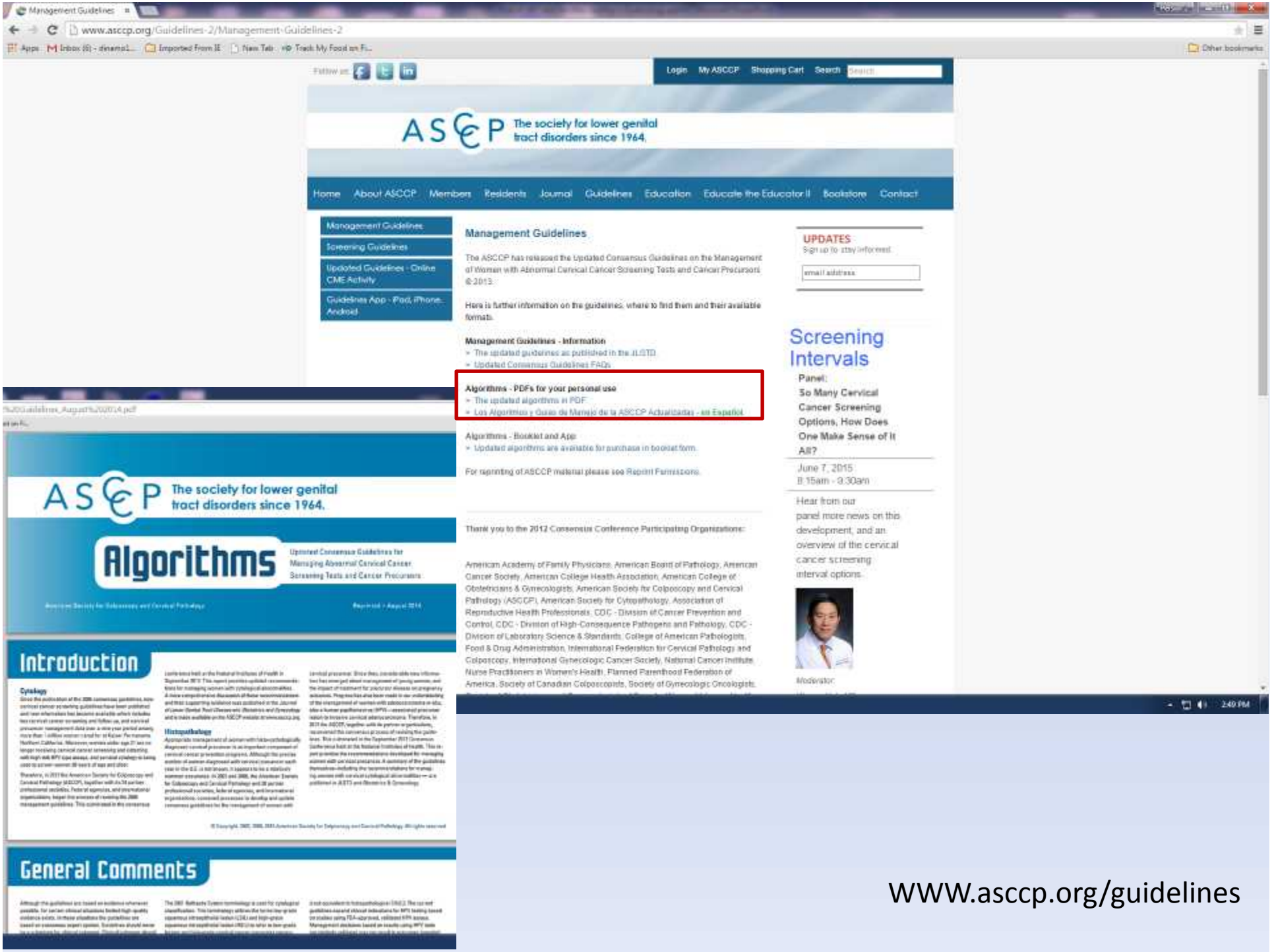
- Co-testing
- Primary Screening
- Quality Improvement
- Risk Stratification and management

ALTS Trial and HPV testing

- **HPV testing at least as sensitive as immed. colposcopy in detecting CIN3+ (about 95%)**
  - **– 55% referred to colposcopy**
- **About 8-9% of ASC-US have CIN3+ after 2 yr. F/U; HPV arm detects most earliest**
- **Need 2 repeat Paps to get same sensitivity**
  - **– Pap specificity worse (colposcopy referral)**
- **HPV/Pap specificity higher in women>29**

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**



WWW.asccp.org/guidelines

ASCCP Guidelines:ASC-US

- **HPV Triage is one of 2 options for ASC-US**
  - **– Others: repeat Pap in a year**
  - **– HPV triage preferred for liquid cytology**
- **Use high-risk HPV panel only**
- **If HPV negative, routine screen Q3 yrs**
- **If HPV positive, refer to colposcopy**
- **Cost-effective approach to ASC-US**

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

HPV and Pap co-testing

- Beginning at age 30
- If double negative, then extended screening interval (5yrs in USA)
- 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 prevalance

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…In press 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

Role of HPV testing in Cervicovaginal cytology

- Triage
  - ASC-US
  - LSIL
  - Secondary triage
- Co-testing
- Primary Screening
- Quality Improvement
- Risk Stratification and management

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

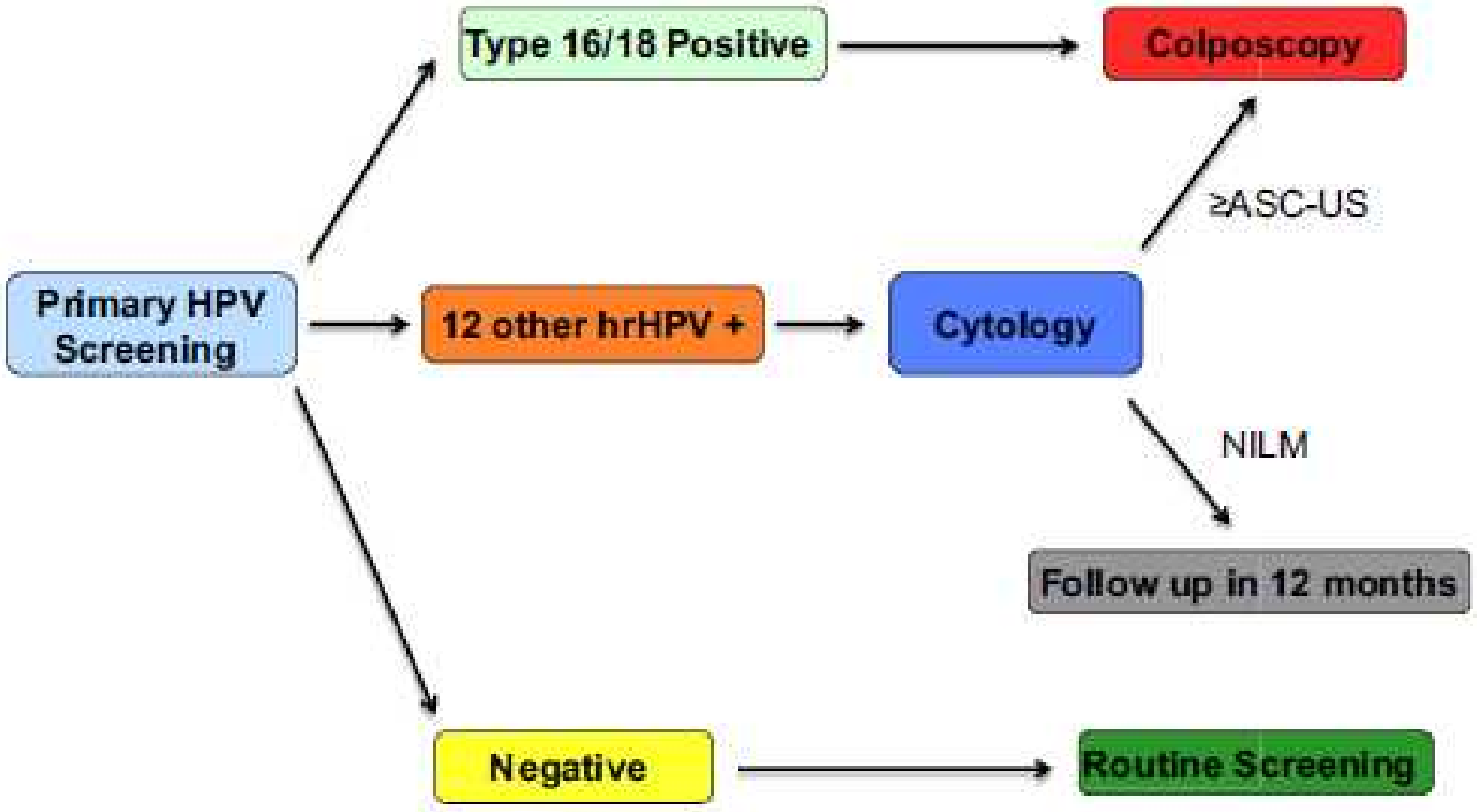


Fig. 1. Recommended primary HPV screening algorithm.

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, 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 6^{th} decade in the USA

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

HPV Positivity Rates in Percentiles (CAP 2013 Data)

Category	Mean (%)	10th	25th	50th	75th	90th
ASC-US	37.1	11.8	26.4	38.3	47.8	54.7
ASC-H	39.9	0	1.0	53.8	68.1	79
NILM>30	10.9	2.1	4.4	6.5	11	22.5
AGC	16.5	0	0	13.2	27	39.3

CAP survey in Archives of Pathology and Lab Medicine, 2015 PMID 25436905

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.
- Nascimento AF, Cibas ES. The ASC/SIL ratio for cytopathologists as a quality control measure: a follow-up study. Am J Clin Pathol. 2007 Oct;128(4):653-6. PubMed PMID: 17875518.
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- 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.

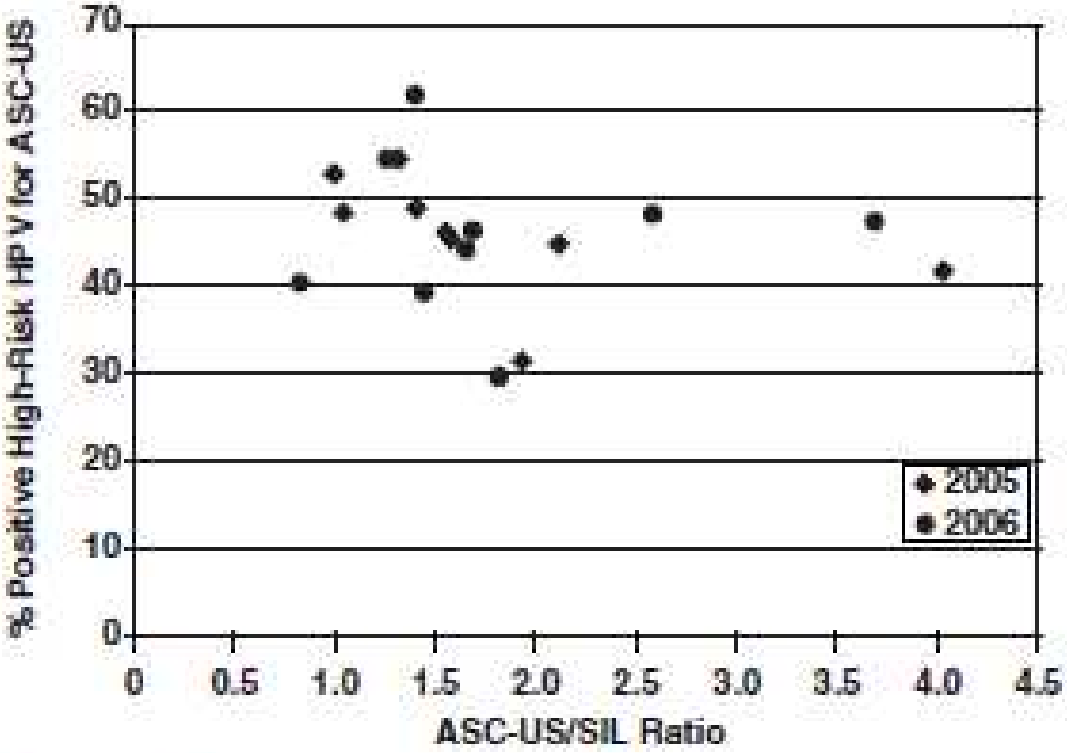

Figure 3 Scatter plot of atypical squamous cells of undetermined significance (ASC-US)/squamous intraepithelial lesion (SIL) ratio and corresponding percentage of ASC-US interpretations that were positive for high-risk human papillomavirus (HPV) DNA for each of 9 cytopathologists in 2005 (diamonds) and 2006 (circles).

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.

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

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*

Examples of In Vitro Molecular Diagnostic Assays for out of Vial Testing From Residual Liquid Based Pap Test Samples

Analyte	Sample	Method	Regulatory Status
HPV	ThinPrep SurePath and others	Hybrid capture II Cervista Cobas Aptima	FDA cleared
Chlamydia Gonorrhoea	ThinPrep SurePath	Amplicor or Aptima	FDA cleared
Herpes Simplex Virus	Residual from liquid based vial	PCR	CLIA validation
Group B Strep	Same as above	Amplification	CLIA validation
Trichomonas	Same as above	Hybridization	FDA or CLIA Validation
Cystic Fibrosis	Same as above	PCR	CLIA Validation

Prophylactic HPV vaccines (First Generation)

- Current vaccines are highly purified virus-like particles (VLPs) of major capsid protein L1
- Produced as recombinant proteins
- L1 VLPs morphologically and immunologically similar to HPV virons but without a viral genome, hence non infectious
- Highly efficacious but protection specific to HPV type

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	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%

Prophylactic HPV vaccines (First Generation)

- Some cross protection against closely related high-risk non-vaccine HPV types like HPV 31(79%),33(46%) and 45(76%) (Cervarix)
- Routine vaccination of girls at 11-12 yrs. as well as unvaccinated women 13-26 yrs. (as early as 9 yrs.)(9-26 yrs)
- Vaccination for males has also been approved

Prophylactic HPV vaccines (Second Generation)

- Based on minor capsid protein L2
- Elicit response against a broad spectrum of HPVs
- Antibody responses against linear L2 epitopes, hence peptide based and cheaper to produce
- Longer shelf life
- Animal model and Human studies very promising
- Remains to be seen what happens clinically once widely implemented

Therapeutic Vaccines

- None ready for current application
- Several approaches to induce Cytotoxic T cell response to HPV E6/E7 pathway
- Bacterial or viral vector based approaches
- Peptide based approaches
- Protein based vaccines safer but less immunogenic
- Dendritic cell-based vaccines
- Cost will be major factor for low resource settings

APMIS ACTA PATHOLOGICA, MICROBIOLOGICA ET IMMUNOLOGICA SCANDINAVICA

APMIS 122: 667–673

DOI 10.1111/apm.12279

REVIEW ARTICLE

Cervical cancer screening at crossroads

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Lynge E, Rygaard C, Baillet MV-P, Dugué P-A, Sander BB, Bonde J, Rebolj M. Cervical cancer screening at crossroads. APMIS 2014; 122: 667–673.

Cervical screening has been one of the most successful public health prevention programmes. For 50 years, cytology formed the basis for screening, and detected cervical intraepithelial lesions (CIN) were treated surgically to prevent progression to cancer. In a high-risk country as Denmark, screening decreased the incidence of cervical cancer from 34 to 11 per 100 000, age-standardized rate (World Standard Population). Screening is, however, also expensive; Denmark (population: 5.6 million) undertakes close to half a million tests per year, and has 6–8 CIN-treated women for each prevented cancer case. The discovery of human papillomavirus (HPV) as the cause of cervical cancer dramatically changed perspectives for disease control. Screening with HPV testing was launched around 1990, and preventive HPV vaccination was licensed in 2006. Long-term randomized controlled trials (RCT) demonstrated that HPV testing provides better protection against cervical cancer than cytology, but it requires extra repeated testing. HPV vaccination RCTs, furthermore, have proved that HPV vaccination protects against vaccine-type high-grade CIN in women vaccinated prior to sexual activity, but less so in women vaccinated later. The challenge now is therefore to find an algorithm for screening of a heterogeneous population including non-vaccinated women; women vaccinated prior to start of sexual activity; and women vaccinated later.

Key words: Cervix uteri; cancer; screening; cytology; human papillomavirus.

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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

Facts Now…Vaccines

- Vaccines currently unaffordable in low resource settings
- Most women beyond recommended age for vaccines
- Vaccines do not protect women already infected before vaccination
- Vaccines do not protect against all HPV types
- Therapeutic vaccines not in the near future and affordability issues

Cervical Cancer Screening

- Will depend upon resources…
- In the high resource setting, some combination of Pap and /or HPV for the next 20+ years for unvaccinated group
- Continue screening vaccinated ones but strategy may have to change
  - With drop in disease prevalence, pap may not be as sensitive
  - ? Pap + P16/ki 67
  - Or HPV primary screen with pap/P16/ki67 triage?