

HPV Testing For Cervical Cancer Screening

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Objectives

- Understand the role of HR-HPV in cervical cancer development
- Know the use of HR-HPV testing in current cervical cancer screening guidelines
- Be able to compare testing principles used in current FDA-cleared HR-HPV assays

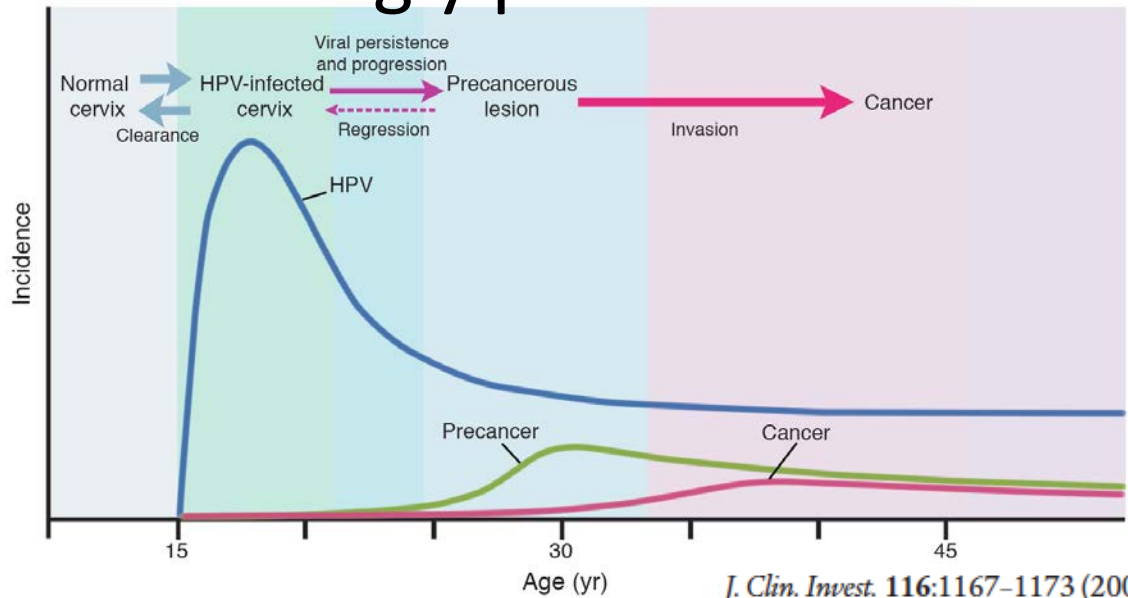
Cervical Cancer

- Most frequent --> #14 (cancer death in women)
 - 12,000 cases, 4,200 deaths, (50% unscreened)
 - Goal: detection of preinvasive disease
 - Early detection: 5-year survival rate >90%
- Persistent HR HPV infection
 - Almost 100% of cervical cancers HR HPV+
 - HPV16 (55-60%), HPV18 (10-15%)
- Cause all common/most rare histologic types
 - Squamous cell carcinoma (80-90%)

Am J Clin Pathol 2012;137:516-542

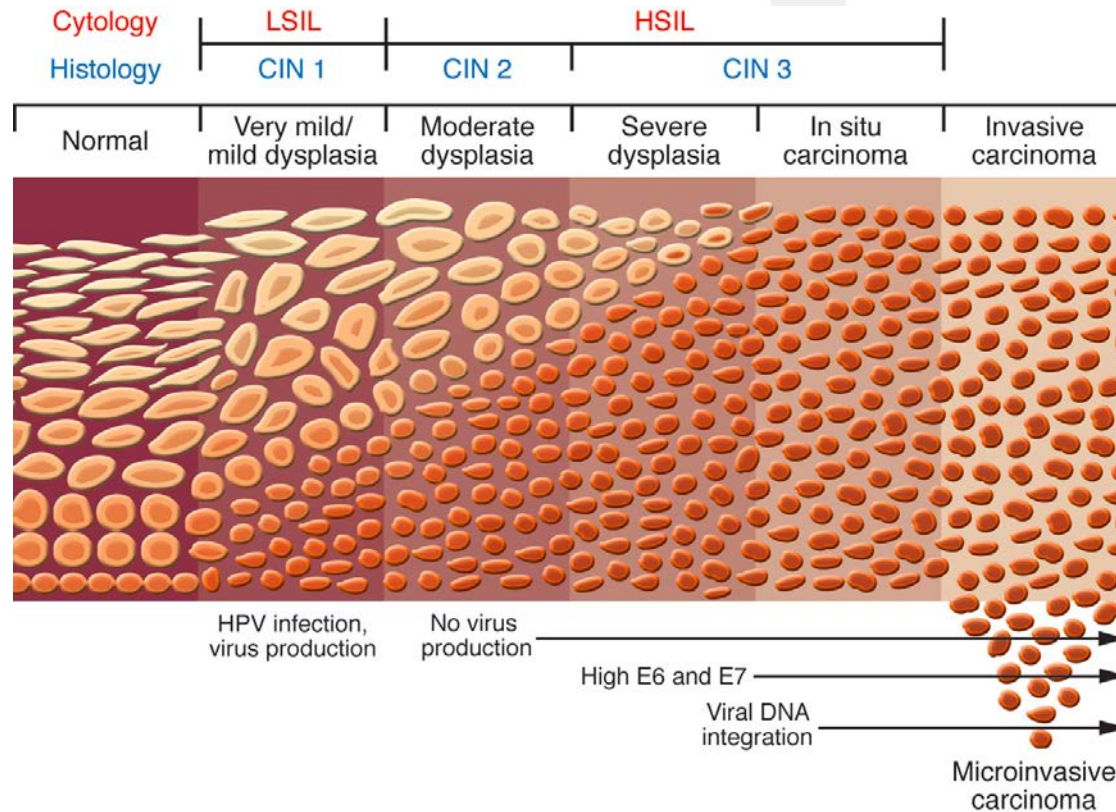
Cervical Carcinogenesis

- Sexual/genital skin-to-skin contact
- Peak: few years after median sexual debut
- 90% clearance within 1-2 years
- 1 & 2-year persistence strongly predicts CIN3+
- Progression
- Invasion



J. Clin. Invest. 116:1167–1173 (2006)

Squamous Cervical Precursor Lesions



- LSIL: Low-grade squamous intraepithelial lesion
~ koilocytic atypia (HPV) and/ or CIN1
- HSIL: High-grade squamous intraepithelial lesion
~ CIN2/3

J Clin Invest 2006;116:1167-1173

Natural History of Cervical Precancer

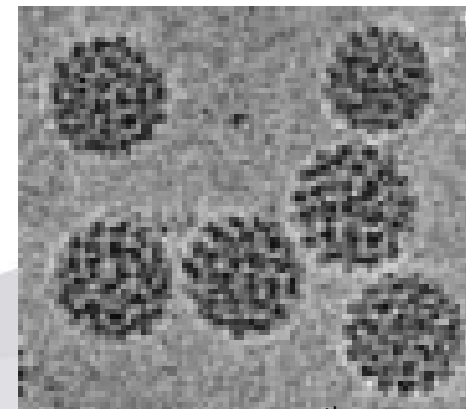
Degree of Dysplasia	Regression (%)	Persistence (%)	Progression to CIN3 (%)	Progression to Invasive Cancer (%)
CIN I	60	30	10	1
CIN II	40	40	15	5
CIN III	33	55	N/A	>12*

* untreated: 30% over 30-year period
treated: 1% over 30-year period

Am J Clin Pathol 2012;137:516-542
Int J Gynecol Pathol 1993; 12(2): 186-92

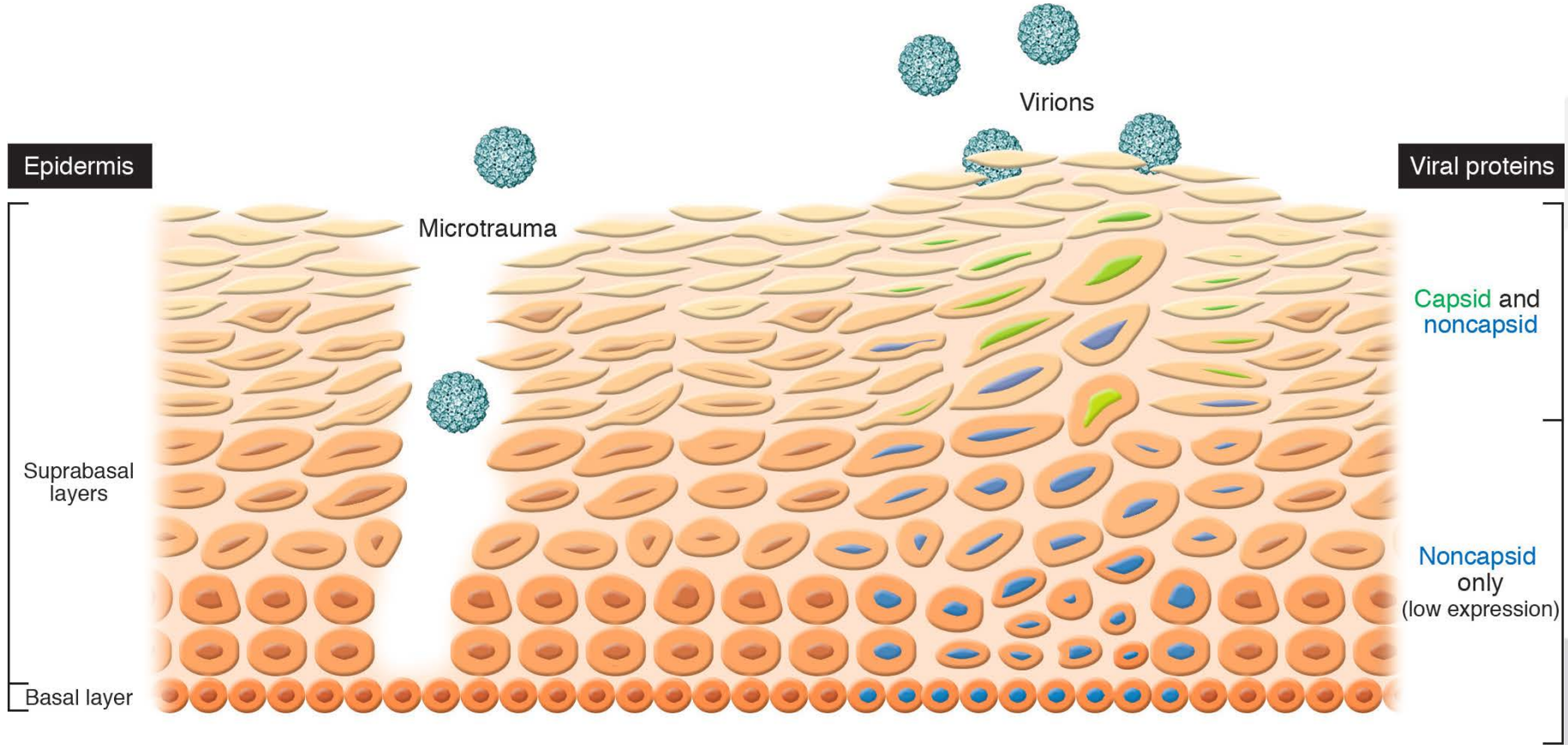
HPV - Biology

- Double-stranded, circular DNA, ~8kb
- Oncogenes (E6, E7)
- >100 types (~40 infect genital tract)
 - **Low risk** (condyloma acuminata): **6, 11**, 42, 43, 44, 54, 61, 70, 72, and 81
 - **High risk** (cervical dysplasia/cancer): **16, 18, 31, 33**, 35, 39, **45**, 51, 52, 56, 58, 59, 68, (73, 82)
 - **Indeterminate risk**
- Squamous epithelium
 - Skin, cervix, larynx, oropharynx, anus, esophagus, conjunctiva



Fields Virology, 5th Edition

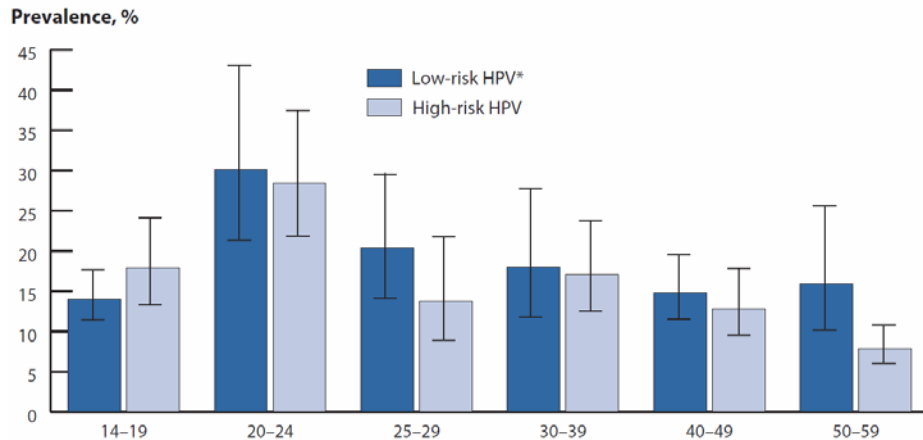
HPV Replication



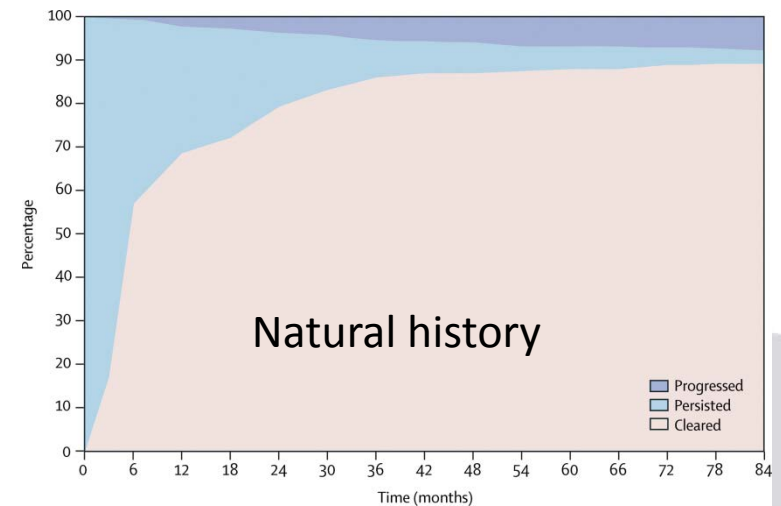
J Clin Invest 2006;116:1167-1173

HPV Infection

- Most common viral STI
- Incidence ~ 6 million/y; prevalence ~20 million
- Lifetime risk ~ 50-75%
- Clearance 70% at 1 yr, 90% at 2 yrs



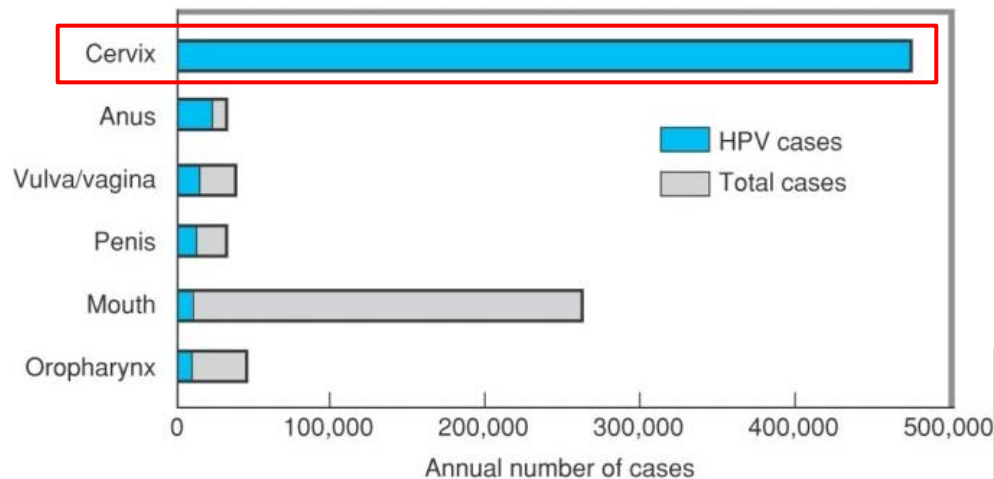
CDC: Sexually Transmitted Disease Surveillance, 2009



Lancet 2007; 370: 890-907

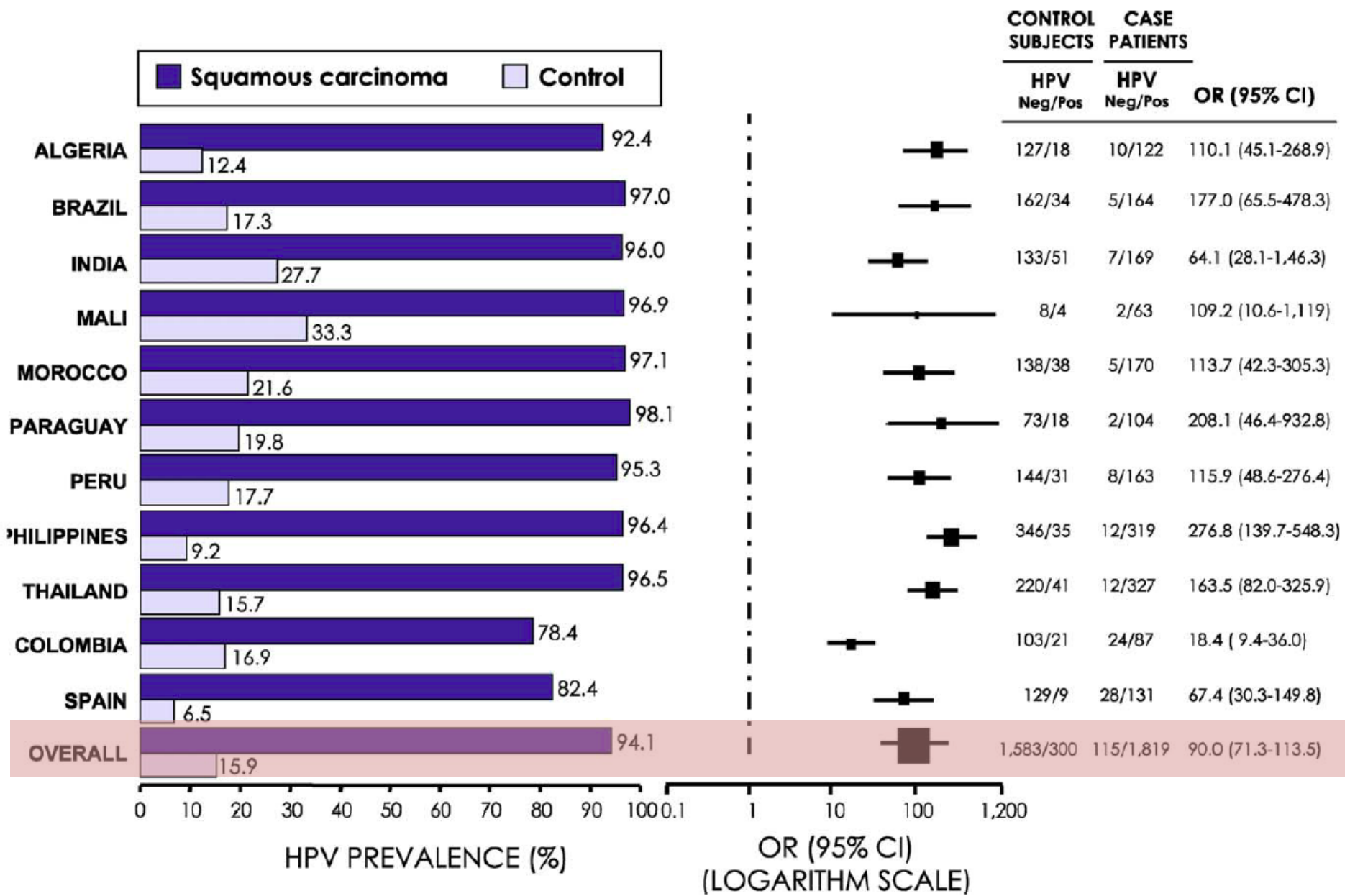
HPV – Pathogenic Spectrum

- **HR HPV** - Squamous cell carcinoma
 - Uterine cervix, vulva, vagina, anus, penis
 - Oropharynx (tonsil, base of tongue), esophagus
- **LR HPV**
 - Genital warts
 - Recurrent respiratory papillomatosis
 - Low-grade cervical abnormalities

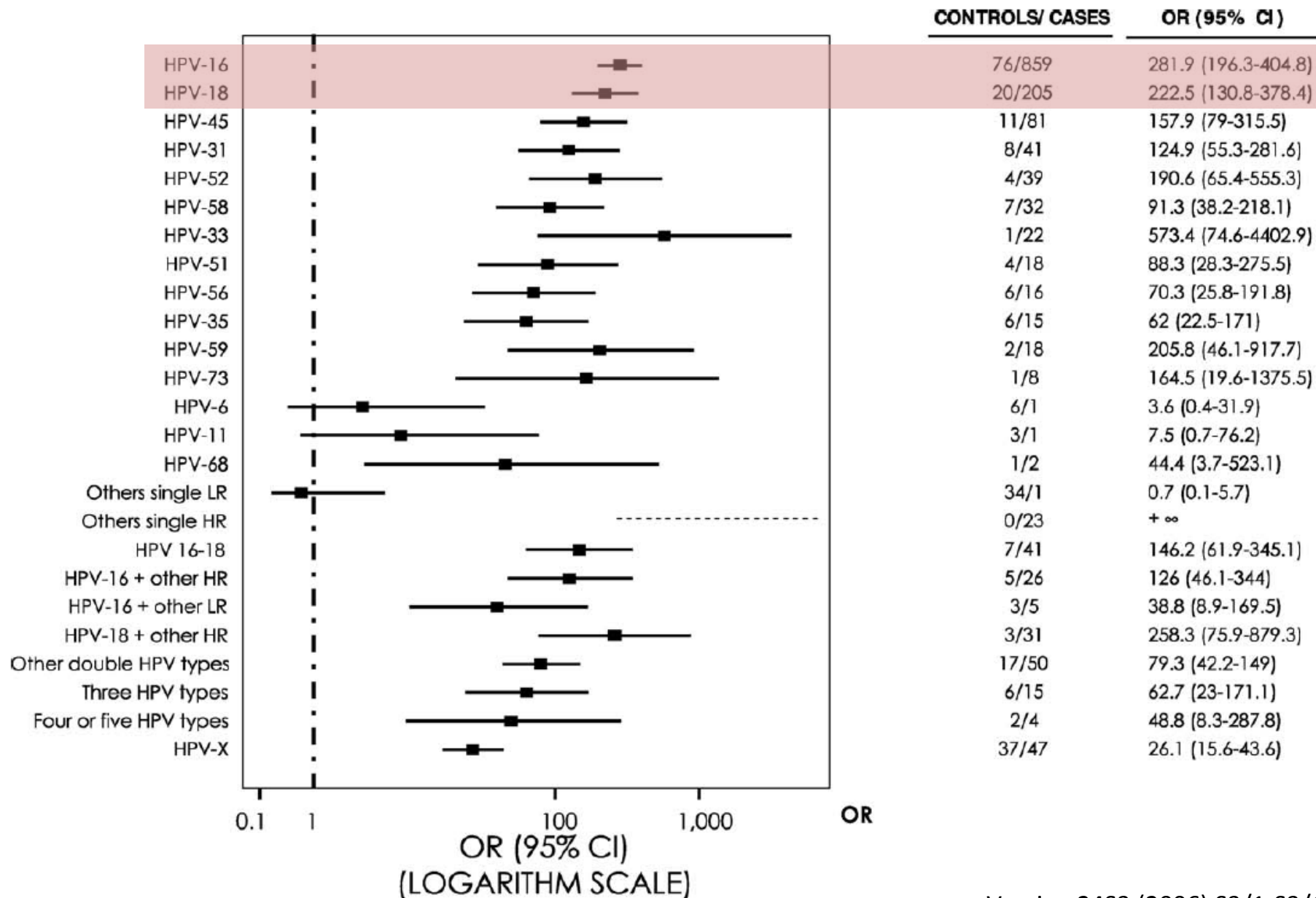


~70%: HPV16 and 18

Fields Virology, 5th Edition



Vaccine 24S3 (2006) S3/1-S3/10



Vaccine 24S3 (2006) S3/1-S3/10

Cervical Cancer Screening

- **Pap test**
 - Identifies **dysplasia / pre-cancer / cancer**
 - Higher **specificity/lower sensitivity**
- **HPV test**
 - Identifies **women at risk**
 - High **negative predictive** value (CIN, cancer)
 - Higher **reproducibility**
- **Combined approach**
 - Co-testing with cytology (≥ 30 y)
 - ASCUS-triage: follow-up interval (≥ 21 y)

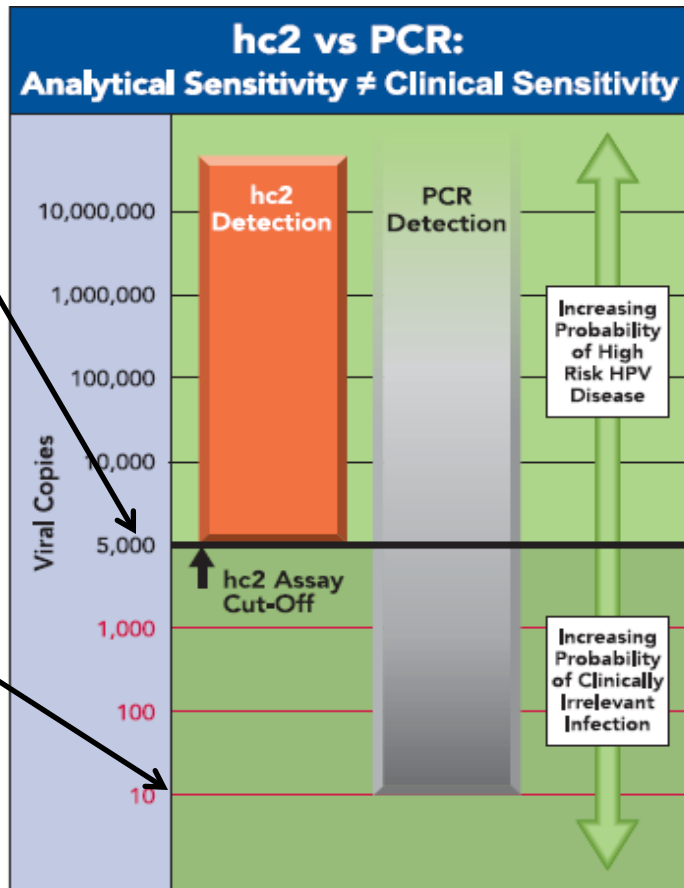
Ann Intern Med 132 (10): 810-9
Am J Clin Pathol 2012;137:516

Clinical Specimens (HC II)

- **Digene Cervical Sampler (Qiagen)**
 - Media optimized for HC2 assay
 - FDA-approved
 - 2 wks (2-30° C), 3 wks (4° C), 3 mo (-20° C)
- **ThinPrep (PreservCyt, 20ml)**
 - 3 weeks from collection (cytology)
 - FDA-approved
 - HCII, Invader, cobas, APTIMA
- **SurePath (TriPath, 10ml)**
 - 4 weeks from collection (cytology)
 - Not FDA-approved
 - Full validation required

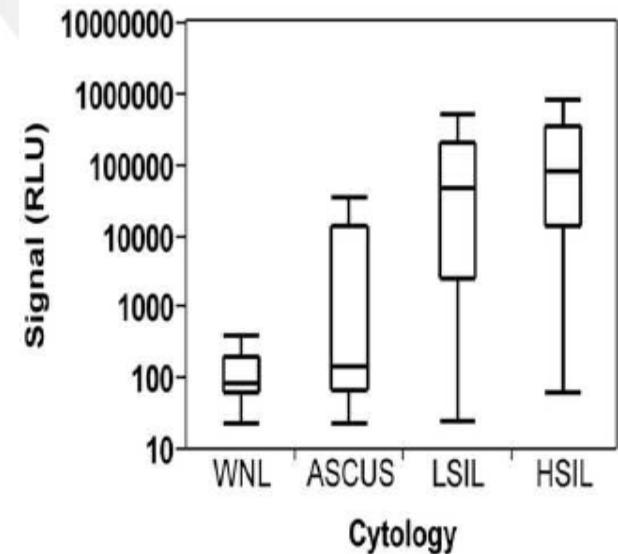


Analytical vs. Clinical Sensitivity



The Analytical Sensitivity of HC2 is 5,000 copies of HPV DNA

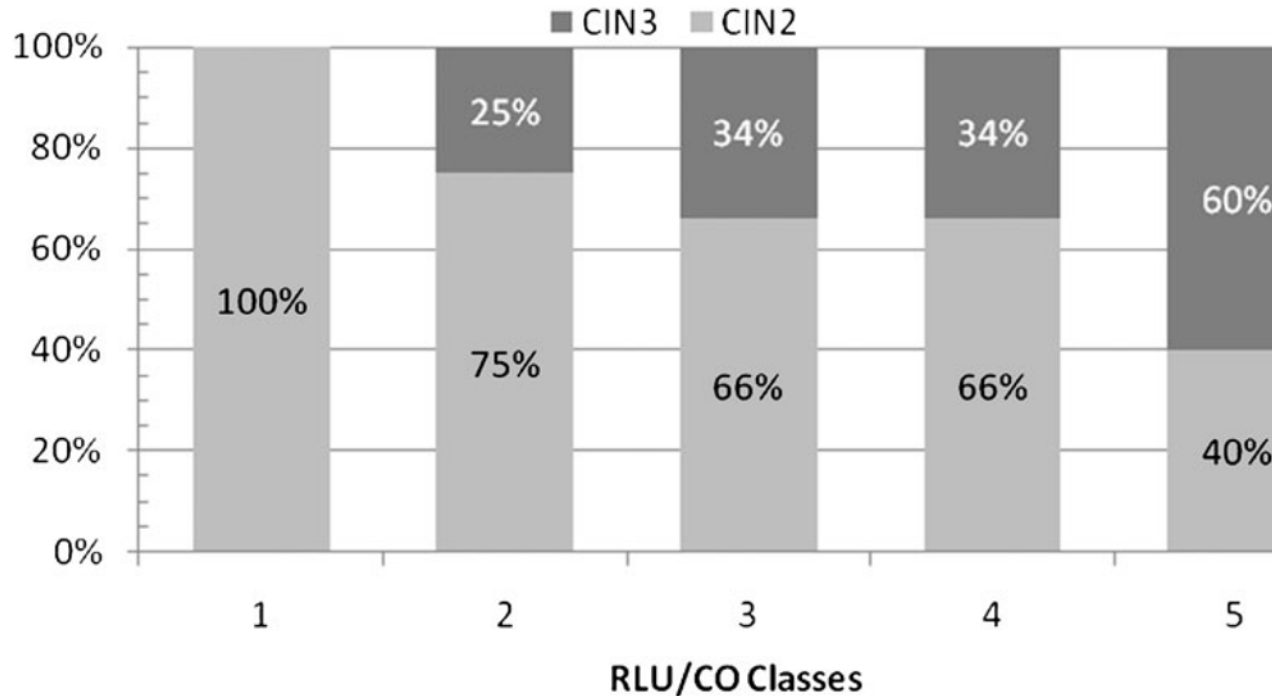
The Analytical Sensitivity of PCR methods can be <10 copies of HPV DNA



J Virol Methods. 2011 Feb 2

Adapted from J Pathol 2003; 201:1-6

Viral Load (RLU) & Histology



1: $0 < \text{RLU/CO values} \leq 1$

2: $1 < \text{RLU/CO values} \leq 10$

3: $10 < \text{RLU/CO values} \leq 100$

4: $100 < \text{RLU/CO values} \leq 1,000$

5: $\text{RLU/CO values} > 1,000$

Eur J Clin Microbiol Infect Dis. 2012 Mar 1

2012 Cervical Screening Guidelines

- American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology
Am J Clin Pathol 2012;137:516-542
- Not applicable for
 - Women with h/o cervical cancer
 - Women with exposure to diethylstilbestrol (DES)
 - Immunocompromised women (e.g. HIV+)
- U.S. Preventive Services Task Force
Ann Intern Med 2012 Mar 14

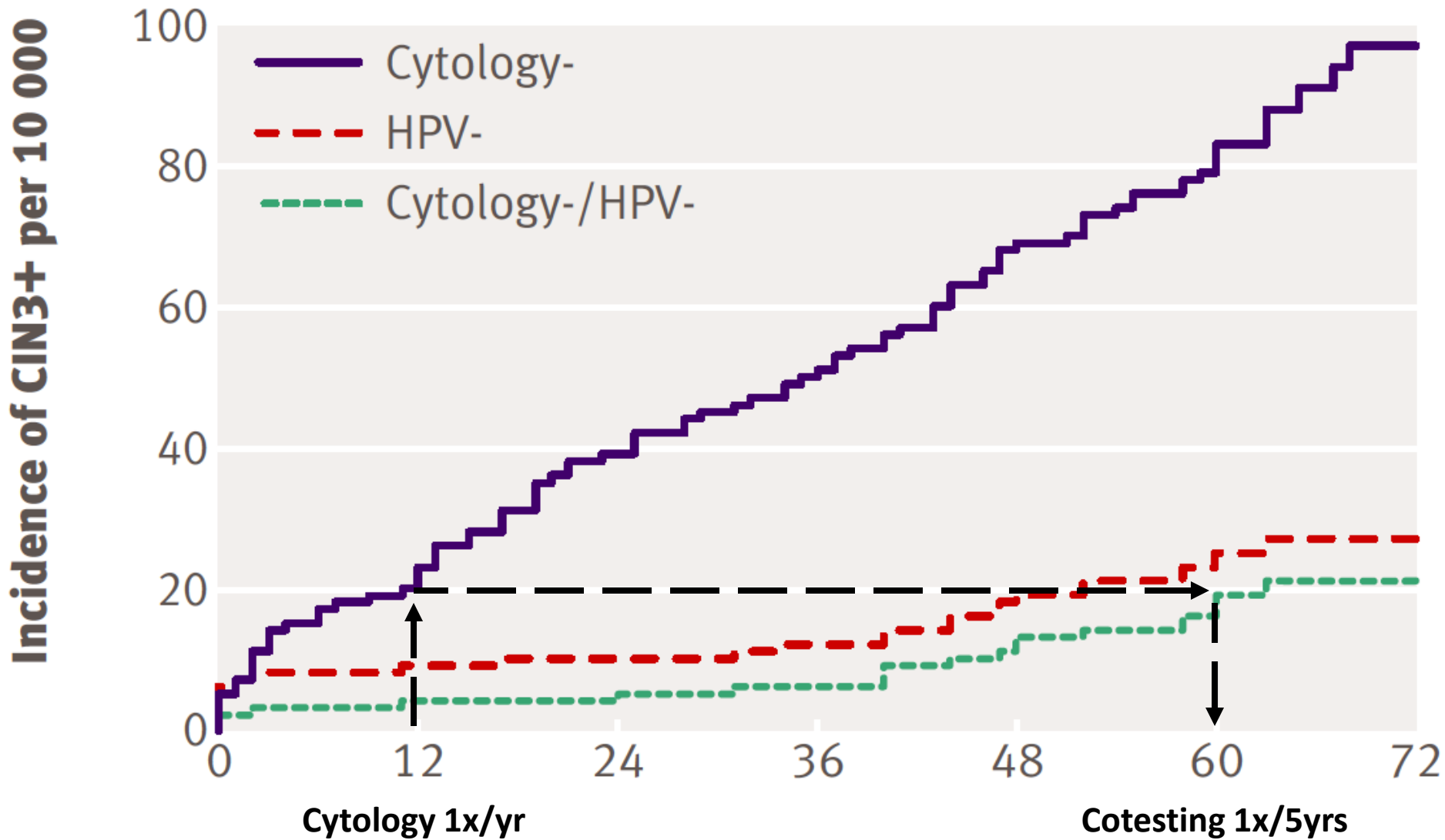
Population	Page Numbers	Recommended Screening Method*	Management of Screen Results	Comments
Aged <21 y		No screening		HPV testing should not be used for screening or management of ASC-US in this age group
Aged 21-29 y		Cytology alone every 3 y	HPV-positive ASC-US [†] or cytology of LSIL or more severe: Refer to ASCCP guidelines ² Cytology negative or HPV-negative ASC-US [†] : Rescreen with cytology in 3 y	HPV testing should not be used for screening in this age group
Aged 30-65 y		HPV and cytology "cotesting" every 5 y (preferred)	HPV-positive ASC-US or cytology of LSIL or more severe: Refer to ASCCP guidelines ² HPV positive, cytology negative: Option 1: 12-mo follow-up with cotesting Option 2: Test for HPV16 or HPV16/18 genotypes <ul style="list-style-type: none"> • If HPV16 or HPV16/18 positive: refer to colposcopy • If HPV16 or HPV16/18 negative: 12-mo follow-up with cotesting Cotest negative or HPV-negative ASC-US: Rescreen with cotesting in 5 y	Screening by HPV testing alone is not recommended for most clinical settings
		Cytology alone every 3 y (acceptable)	HPV-positive ASC-US [†] or cytology of LSIL or more severe: Refer to ASCCP guidelines ² Cytology negative or HPV-negative ASC-US [†] : Rescreen with cytology in 3 y	
Aged >65 y		No screening following adequate negative prior screening		Women with a history of CIN2 or a more severe diagnosis should continue routine screening for at least 20 y
After hysterectomy		No screening		Applies to women without a cervix and without a history of CIN2 or a more severe diagnosis in the past 20 y or cervical cancer ever
HPV vaccinated		Follow age-specific recommendations (same as unvaccinated women)		

ASCCP, American Society for Colposcopy and Cervical Pathology; ASC-US, atypical squamous cells of undetermined significance; CIN2, cervical intraepithelial neoplasia grade 2; HPV, human papillomavirus; LSIL, low-grade squamous intraepithelial lesion.

* Women should not be screened annually at any age by any method.

† ASC-US cytology with secondary HPV testing for management decisions.

Rationale For Screening Interval



Main Points

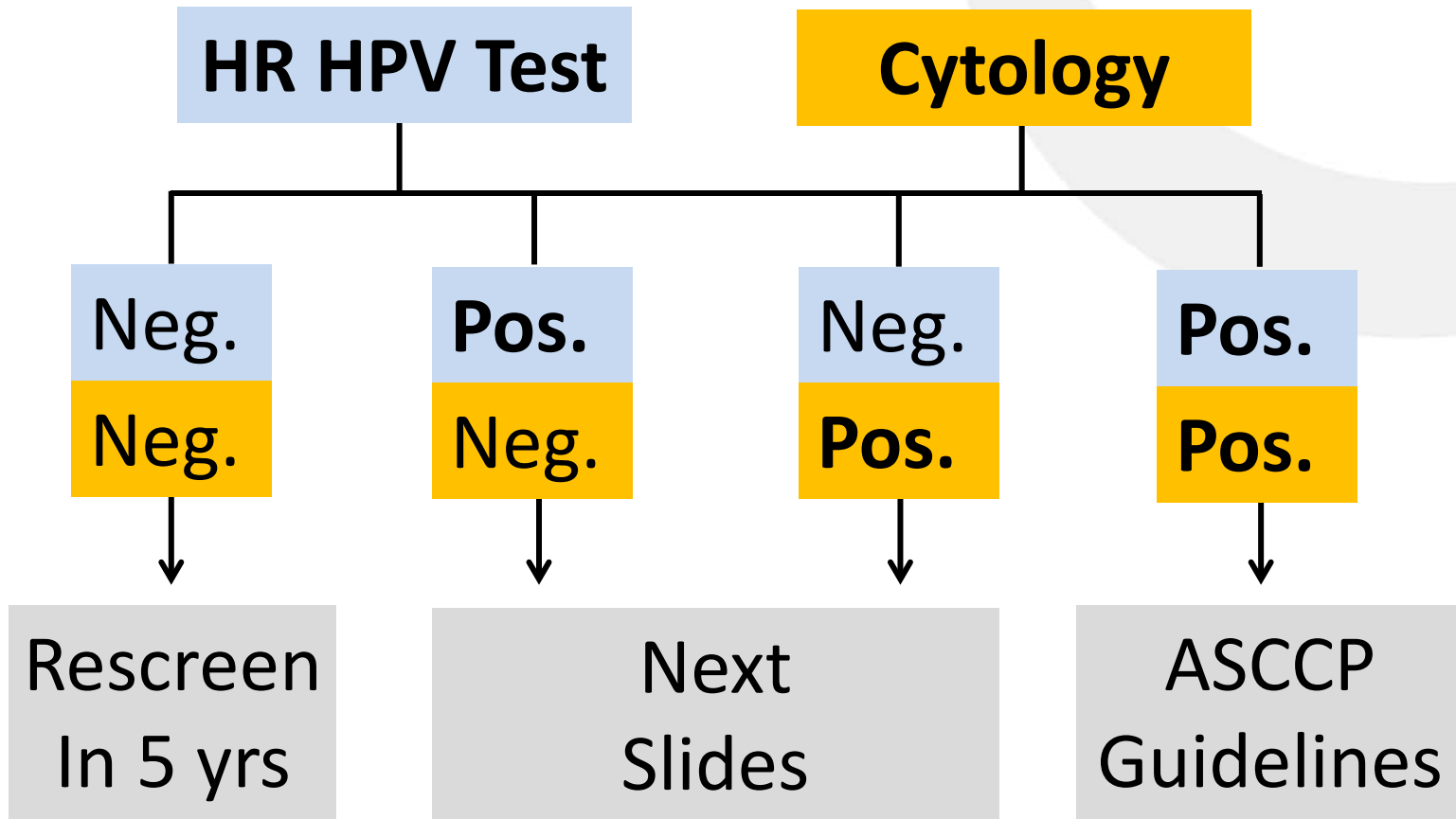
- **No annual screening at any age**
- **Age <21: no screening**
 - No reduction over past 4 decades
 - Prevention through vaccination
- **Age 21-29: cytology q3 yrs, no cotesting**
 - q1 year: 2x colposcopies, slight cancer reduction
- **Age 30-65: cotesting q5 yrs or **cytology q3 yrs****
 - Incident cancer equal/lower with cotesting
 - Cotesting: better detection of adenocarcinoma

Am J Clin Pathol 2012;137:516

Main Points, Cont.

- **Age >65 and no history of CIN2+ in last 20 yrs**
 - **STOP**
- **Age >65 with history of CIN2+ in last 20 yrs**
 - Continue **routine screening** for at least 20 yrs
- **Hysterectomy, no history of CIN2+**
 - **STOP**
- **Vaccinated women**
 - Same

Cotesting Follow-Up



Cytology-**neg** & HPV-**pos**

- **No direct referral to colposcopy**
- **Option 1: repeat cotesting after 12 months**
 - Either test positive (LSIL+) -> colposcopy
 - Both negative -> return to normal screening
- **Option 2: immediate HPV16 ± HPV18**
 - HPV16 and/or HPV18-**positive** -> **colposcopy**
 - HPV16 and 18 **negative** -> cotest **after 12 months**
 - Follow-up see option 1

ASC-US & HPV-neg

- Continue with **routine** screening

ASC-US & HPV-pos

- Direct referral to **colposcopy**

LSIL+ & Irrespective of HPV

- Direct referral to colposcopy

FDA-Cleared HPV Tests

- **Hybrid Capture 2 High-Risk HPV DNA Test (Qiagen)**
 - Probe hybridization (whole genome); HR, (LR)
- **Cervista HPV HR (Hologic)**
 - Invader; HR, IC; HPV16, HPV18
- **cobas HPV Test (Roche)**
 - Real-time PCR; HR, IC; HPV16, HPV18
- **APTIMA HPV Assay (Gen-Probe)**
 - TMA; HR, IC

Hybrid Capture

- RNA probes targeting most of genome, hybrid
- Capture Ab, AP-conjugated mAb
- **Signal** amplification
- 13 HR types targeted
 - Cross-hybridization: HR: 66; LR: 8, 9, 43, 45, 47
- No extraction
- No target amplification
- No internal control

Invader

- DNA extraction
- Probes for 14 HR HPV types
 - Probe and Invader oligonucleotides anneal
 - Cleavase: overlapping probes
 - Release of 5' flap
 - Flap + FRET probe -> signal
- **Signal** amplification
- No target amplification
- Internal control (histone 2)

Real-Time PCR

- DNA extraction
- Primers/probes for 14 HR types
- Multiple primer/probe sets
- **Target** amplification
- Internal control (β -globin)
- Optional/simultaneous: HPV16/18 typing

TMA

- E6/E7 mRNA
- 14 HR types
 - Target capture, amplification (TMA), detection (hybridization protection assay)
- **Target** amplification
- Internal control
- Separate RUO assay: HPV16 vs. 18/45

Performance and Workflow

- Sample volume requirements
- Prequot vs. postquot samples
- Sample preservative
- Screening vs. triage
- Clinical sensitivity and specificity
- Throughput
- Automation vs. manual steps
- Cross-contamination risk

One Test Comparison Study

- Referral population, n=1099
- CIN2+ n=359 (33%), CIN3+ n=224 (20%)
- ThinPrep samples

Table 2: HPV positivity and type-specific results of different tests

Test:	Number tested (Number with single mild or less smear)	positive (%)	HPV 16 positive (%)	HPV 18 positive (%)	Other HPV positive (%)*
Qiagen: Hybrid Capture 2	1067 (649)	85.8	-	-	-
Roche: Cobas	1095 (670)	82.3	31.9	10	71.1
Abbott: Real-time PCR	1095 (670)	79.4	30.5	8.5	67.6
BD HPV	1097 (670)	82.0	30.5	9.4	71.4
Gen-probe: APTIMA	1097 (670)	79.0	30.0	11.8**	-
Norchip: PreTect HPV-Proofer	1057 (641)	43.9	26.2	7.8	42.8
mtm laboratories: p16 ^{INK4a}	974 (591)	58.9	-	-	-

* Other HPV only for those negative for HPV 16 and HPV 18

** tested HPV 18/45 combined

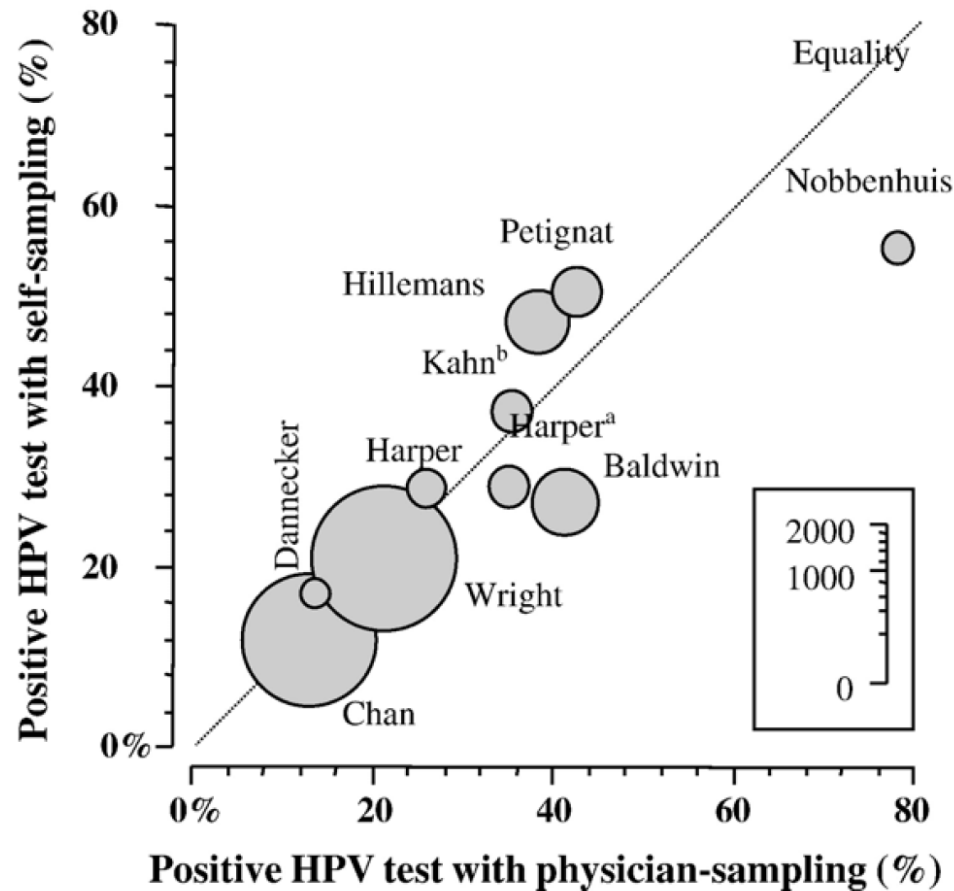
Test (no. assessed)	All women		
	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)
Qiagen: Hybrid Capture 2			
CIN3+	98.7 (96.1-99.7)	-	24.0 (21.3-26.9)
CIN2+	96.3 (93.8-98.0)	19.5 (16.7-22.6)	37.4 (34.2-40.6)
CIN2	92.4 (86.5-96.3)	-	-
Roche: Cobas			
CIN3+	97.3 (94.2-99.0)	-	23.9 (21.1-26.8)
CIN2+	95.2 (92.5-97.2)	24.0 (20.9-27.2)	37.6 (34.5-40.9)
CIN2	91.9 (85.9-95.9)	-	-
Abbott: Real-time PCR			
CIN3+	97.3 (94.2-99.0)	-	24.7 (21.9-27.8)
CIN2+	93.3 (90.1-95.6)	27.3 (24.1-30.7)	38.2 (35.0-41.5)
CIN2	86.7 (79.7-91.9)	-	-
BD HPV			
CIN3+	97.8 (94.8-99.3)	-	24.2 (21.5-27.2)
CIN2+	95.0 (92.2-97.0)	24.2 (21.2-27.5)	37.8 (34.6-41.0)
CIN2	90.4 (84.1-94.8)	-	-
Gen-Probe: APTIMA			
CIN3+	97.8 (94.8-99.3)	-	25.1 (22.3-28.2)
CIN2+	95.3 (92.5-97.2)	28.8 (25.6-32.2)	39.3 (36.1-42.7)
CIN2	91.1 (85.0-95.3)	-	-
mtm laboratories: p16 ^{INK4a}			
CIN3+	90.2 (85.3-93.9)	-	32.2 (28.4-36.2)
CIN2+	85.7 (81.5-89.3)	54.7 (50.8-58.6)	49.1 (45.0-53.3)
CIN2	78.2 (69.9-85.1)	-	-
Norchip: PreTect HPV-Proofer			
CIN3+	80.3 (74.4-85.3)	-	37.7 (33.3-42.3)
CIN2+	74.1 (69.1-78.6)	70.8 (67.3-74.2)	55.4 (50.7-60.0)
CIN2	63.6 (54.6-71.9)	-	-
Cytology (mild or worse)			
CIN3+	92.9 (88.7-95.9)	-	33.1 (29.4-36.9)
CIN2+	88.9 (85.1-91.9)	58.1 (54.5-61.7)	50.7 (46.7-54.7)
CIN2	82.2 (74.7-88.3)	-	-

Women with referral less or equal to single mildly dyskaryosis			
Test (no. assessed)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)
Qiagen: Hybrid Capture 2			
CIN3+	100.0 (92.7-100.0)	-	9.2 (6.9-11.9)
CIN2+	93.0 (86.8-96.9)	19.9 (16.5-23.5)	20.0 (16.7-23.6)
CIN2	87.9 (77.5-94.6)	-	-
Roche: Cobas			
CIN3+	100.0 (92.7-100.0)	-	9.3 (7.0-12.2)
CIN2+	94.9 (89.2-98.1)	25.1 (21.6-29.0)	21.1 (17.7-24.9)
CIN2	91.2 (81.8-96.7)	-	-
Abbott: Real-time PCR			
CIN3+	100.0 (92.7-100.0)	-	9.8 (7.3-12.7)
CIN2+	92.3 (85.9-96.4)	28.9 (25.2-32.9)	21.6 (18.0-25.4)
CIN2	86.8 (76.4-93.8)	-	-
BD HPV			
CIN3+	100.0 (92.7-100.0)	-	9.4 (7.0-12.2)
CIN2+	94.0 (88.1-97.6)	25.3 (21.7-29.2)	21.0 (17.6-24.8)
CIN2	89.7 (79.9-95.8)	-	-
Gen-Probe: APTIMA			
CIN3+	100.0 (92.7-100.0)	-	9.8 (7.3-12.7)
CIN2+	92.3 (85.9-96.4)	29.1 (25.4-33.1)	21.6 (18.1-25.5)
CIN2	86.8 (76.4-93.8)	-	-
mtm laboratories: p16 ^{INK4a}			
CIN3+	83.7 (69.3-93.2)	-	12.5 (8.9-16.9)
CIN2+	76.2 (66.9-84.0)	57.2 (52.7-61.6)	27.8 (22.7-33.3)
CIN2	71.0 (58.1-81.8)	-	-
Norchip: PreTect HPV-Proofer			
CIN3+	80.9 (66.7-90.9)	-	16.6 (12.0-22.1)
CIN2+	73.6 (64.4-81.6)	72.1 (68.1-75.9)	35.4 (29.2-41.9)
CIN2	68.3 (55.3-79.4)	-	-
Cytology (mild or worse)			
CIN3+			
CIN2+		N/A	
CIN2			

Future Issues

- Vaginal self sampling (screening uptake)
 - *Gynecologic Oncology* 105 (2007) 530–535
 - *J Natl Cancer Inst* 2012;104:178–188
 - *Lancet* 2011; 378: 1868–73
- Primary screening
 - *J Obstet Gynaecol Can* 2012;34(5):443–452
 - *J Natl Cancer Inst* 2012;104:178–188
 - *BJOG* 2012;119:650–652
 - *Lancet Oncol* 2010; 11: 249–57
- Screening and prognostic testing in other cancers

Self-Collected Vaginal Samples



Gynecol Oncol 105(2007):530

Questions?



Oropharyngeal SCC & HPV Testing

- >36,000 cases/y
- >7,000 deaths/y
- RF: tobacco, alcohol <-> HPV
- HPV-associated
 - Increasing, mostly oropharyngeal
 - Younger, often without tobacco/alcohol
 - Better response to radiation, better survival
 - HPV 16 >> HPV 18

Increasing Incidence

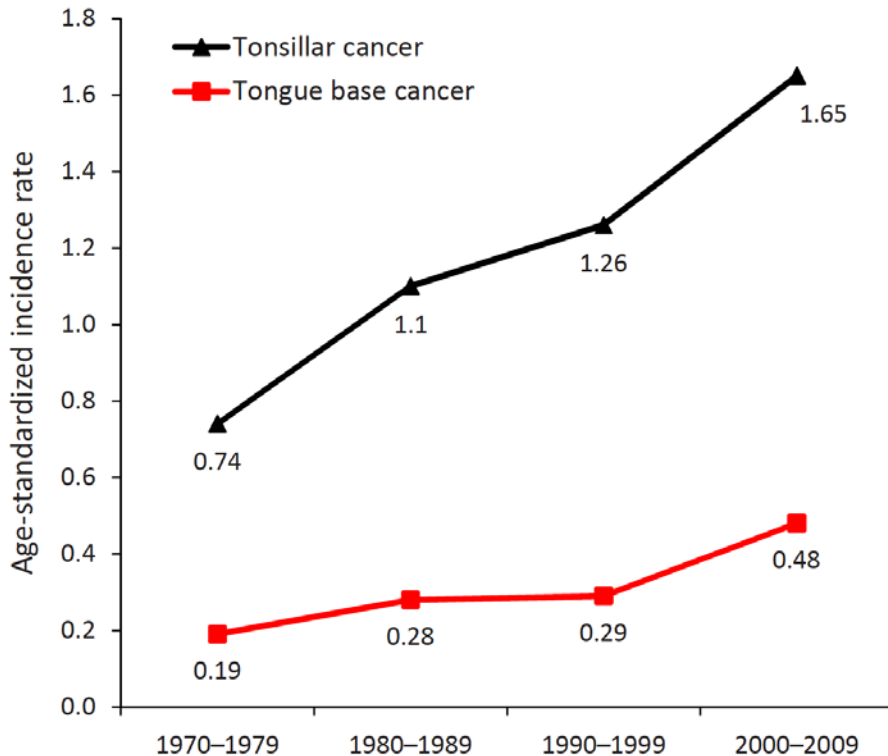


Figure 2. Age-standardized incidence of tonsillar and base of tongue cancers, Stockholm, Sweden, 1970–2006.

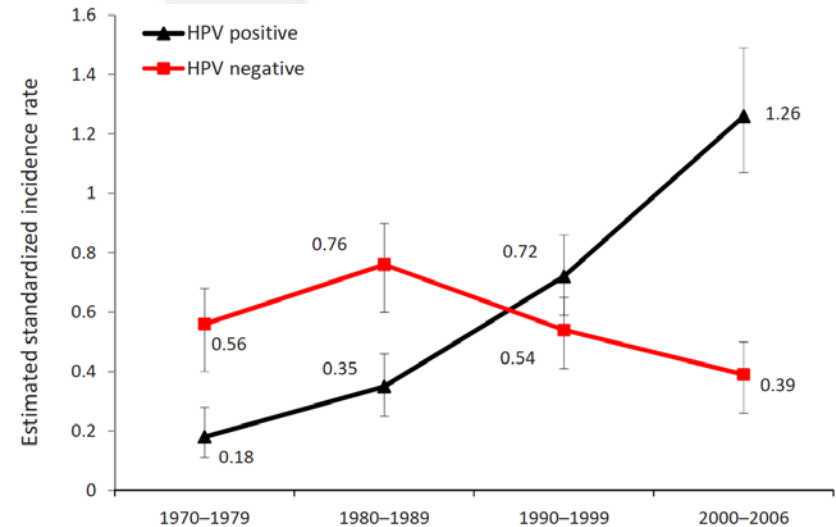
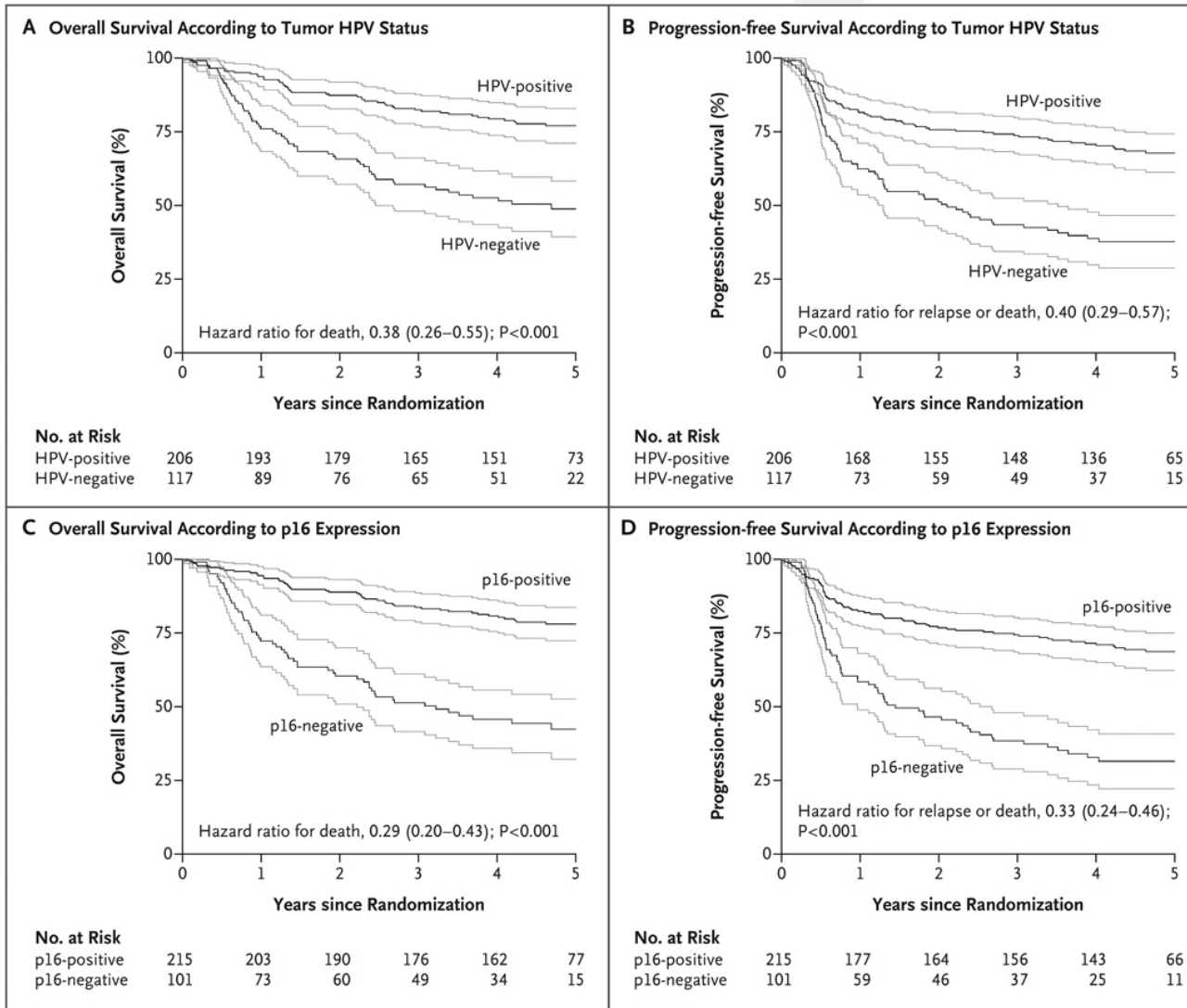


Figure 3. Estimated age-standardized incidence of human papillomavirus (HPV)-positive and HPV-negative tonsillar cancer squamous cell carcinoma cases per 100,000 person-years, Stockholm, Sweden, 1970–2006. Error bars indicate 95% confidence intervals. Data from Näsman et al. (18), with permission of John Wiley and Sons (www.interscience.wiley.com).

HPV In Oropharyngeal Cancers



N Engl J Med. 2010 Jul 1;363(1):24-35

Open Questions

- Specimen
 - Cytology specimens (FNA, brush)
 - Surgical specimens
- Modality
 - In-situ hybridization for HPV
 - Molecular testing for HPV (E6 mRNA)
 - Immunohistochemistry for p16

Questions?

