

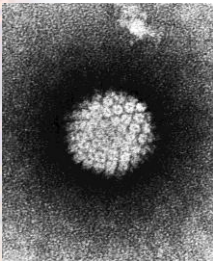
## Update: Molecular Diagnosis of Human Papilloma Virus Infections

Ted E. Schutzbank, PhD, (DABMM)  
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Covance Central Laboratory Services

## Human Papilloma Virus



## Human Papilloma Virus



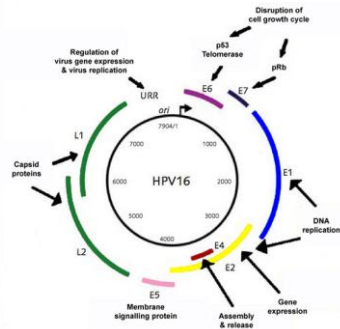
- Member of the Papovavirus family
- Small, Icosahedral particles
- 52 – 55 nm in diameter
- Double-stranded circular genome
  - 8 KBase pairs
  - Complete gene sequence is available

[http://commons.wikimedia.org/wiki/Image:Papilloma\\_Virus\\_\(HPV\)\\_EM.jpg](http://commons.wikimedia.org/wiki/Image:Papilloma_Virus_(HPV)_EM.jpg)

## HPV Lifecycle



## HPV – Genomic Organization



<http://www.microbiologybytes.com/virology/Papillomaviruses.htm>

## HPV Proteins

Early region	Protein functions
E1	Unwinds the DNA strands working with E2 protein Modulate the transcription activity of the E2 protein
E2	Enables E1 protein to bind to the viral origin of replication located within the LCR Encodes a LCR-binding protein that regulates transcription of the early region
E4	Encodes a protein that interacts with cytokeratin
E5	Expressed in later stages of infection, when complete virions are being assembled Augment cellular proliferation and DNA synthesis in a context of cell membrane receptors, such as EGF and PDGF
E6	Induces an increase in mitogen-activated protein kinase activity Binds to p53 and targets it for rapid degradation via a cellular ubiquitin ligase Induces telomerase activation
E7	Binds to the hypophosphorylated Rb proteins and liberate E2F, which results in S phase entry Interacts with inhibitors of cyclin dependent kinases Induces abnormal centrosome duplication resulting in aneuploidy

Jo H, Kim, JW. Cancer Therapy 3: 419-435, 2005

## HPV Infections

- Most common sexually transmitted disease in the US
- Most infections are self limiting and asymptomatic
- >200 types of HPV based on genotype and site of infection
  - Mucosal epithelium
  - Cutaneous epithelium

## Diseases caused by HPV

Disease	HPV Type
Common warts	2, 7
Plantar warts	1, 2, 4
Flat warts	3, 10
Anogenital warts	6, 11, 42, 43, 44, 55 and others
Genital Cancers	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, 82
Epidermodysplasia verruciformis	more than 15 types
Focal epithelial hyperplasia	3, 5, 8, 10 (and others)
Oral papillomas	6, 7, 11, 16, 32



## HPV Infection of Mucosal Epithelium

- > 40 different types that infect mucosal epithelium
- Divided into two oncogenic categories
  - Low Risk
  - High risk
    - Cervical carcinoma
    - Anorectal dysplasia and cancer in homosexual men

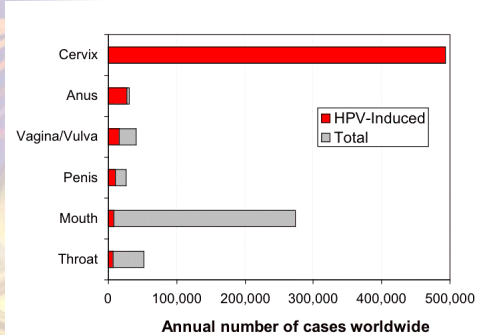
## Symptoms of Genital HPV Infections

- Abnormal vaginal bleeding after sexual intercourse
- Increased dampness or moisture in the area of the lesions
- Itching of the penis, scrotum, anal area or vulva
- Increased vaginal discharge

## Genital HPV Prevalence by Age

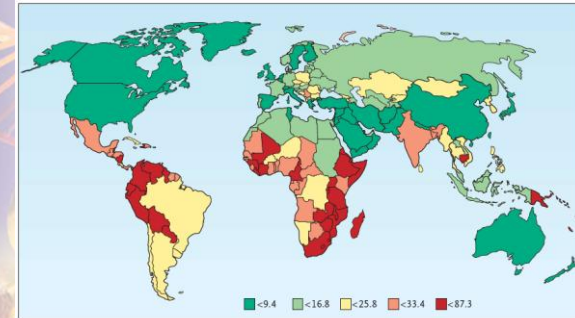
Age (years)	Prevalence (%)
14 – 19	24.5
20 - 24	44.8
25 - 29	27.4
30 – 39	27.5
40 – 49	25.2
50 - 59	19.6
14 - 59	26.8

## HPV and Cancer



Parkin, D. M. Int J Cancer. 2006; 15;118:3030-44

## HPV and Cancer



Incidence of Cervical Cancer Worldwide.  
Numbers indicate cases per 100,000 population.

Schiffman M., Castle P. E. N Engl J Med 2005; 353:2101-2104.

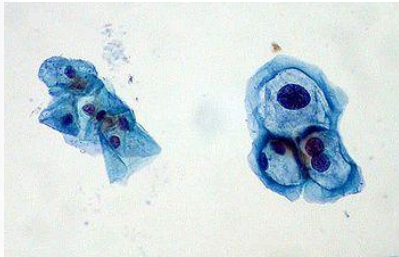
## Risk Factors

- Multiple Sexual Partners
- Early onset of sexual activity
- Tobacco and Alcohol usage
- Stress and other viral infections
  - HSV
  - HIV

## HPV Infection of Mucosal Epithelium

High-risk (oncogenic or cancer-associated) types	Low-risk (non-oncogenic) types
Common types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 82	Common types: 6, 11, 40, 42, 43, 44, 54, 61, 72, 73, 81

## Cervical Cancer

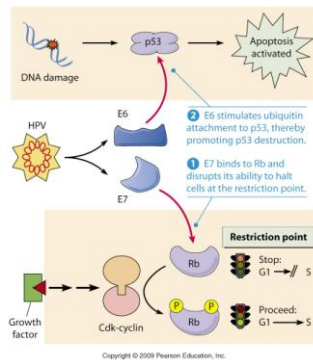


ThinPrep Pap smear with group of normal cervical cells on left and HPV-infected cells on right. The HPV-infected cells show features typical of koilocytes: enlarged (x2 or x3) nuclei and hyperchromasia

## HPV in Cervical Cancer

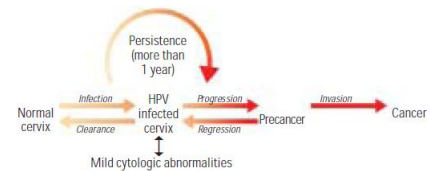
- HPV DNA integrates into host genome
- Integration disrupts E1/E2, genes
- E6/E7 genes overexpressed, resulting in cell transformation

## The Role of E6 and E7



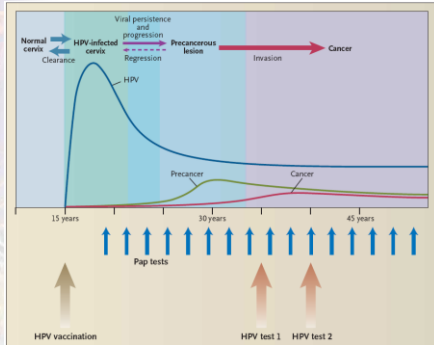
<http://liveonearth.livejournal.com/612978.html>

## Natural History of Cervical Cancer



M. Schiffman, ASCOP, 2002

## The Natural History of HPV Infection and Cervical Cancer



Schiffman M., Castle P. E. N Engl J Med 2005; 353:2101-2104, Nov 17, 2005

## Diagnosis

- No serological diagnosis
- No convenient cell culture
- Cytology
- Histology
- Colposcopy in the case of genital HPV infections.

## Diagnosis

- **Electron microscopy**
  - Only applicable in benign lesions
  - in malignant lesions the viral DNA is integrated and viral particles are not produced
- **Immunocytochemistry**
  - can detect major capsid protein
  - generally group specific not type specific
- **DNA detection techniques**

## Commercially Available HPV DNA Detection Assays

HPV DNA Detection Methods		
Method	Manufacturer	Clinical Utility
In Situ Hybridization	Dako, InnoGenex, Ventana	Direct detection of high risk HPV DNA in tissue biopsies
Hybrid Capture High Risk Types	Digene	Testing for high-risk HPV subtypes is recommended for patients with a cytological finding of ASCUS
Invader HPV ASR	Third Wave Technologies	
PCR	Roche Diagnostics	
Hybrid Capture Low Risk Types	Digene	None

## Commercially Available HPV Nucleic Acid Assays

HPV DNA Genotyping Methods		
Method	Manufacturer	Clinical Utility
Invader HPV16/18*	Hologic	Risk Stratification
PCR/Reverse Line Probe Hybridization	Roche Diagnostics Innogenetics	
INFINITI® HPV Genotyping	AutoGenomics Inc.	

## Indications for HPV DNA Testing

- To screen women with atypical squamous cells of undetermined significance (ASC-US) cervical cytology results to determine the need for referral to colposcopy.
- Used adjunctively with cervical cytology to screen women 30 years and older to assess the presence or absence of high-risk HPV types.

## Indications for HPV DNA Testing

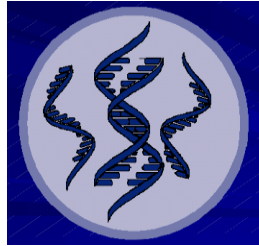
- HPV HR DNA testing should not be used as a screening device for women under age 30 with normal cervical cytology.
- HPV HR DNA testing is not intended to substitute for regular cervical cytology screening.
- Detection of HPV DNA cannot predict persistence of any one type.

## Digene HC 2 Assay

- Two configurations
  - Low Risk HPV
    - 6/11/42/43/44
  - High Risk
    - 16/18/31/33/35/39/45/51/52/56/58/59/68
- Recommended Specimen Types
  - Cervical Swabs in proprietary collection device
  - Liquid Collection media
    - Cytoc ThinPrep (FDA approved)
    - TriPath (BD) SurePath

## Digene Hybrid Capture 2

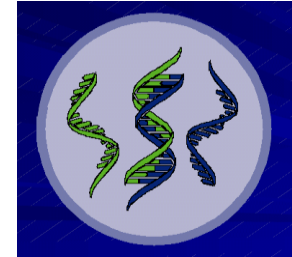
- Release and denature nucleic acids



Images courtesy of Digene Inc. ([www.qiagen.com](http://www.qiagen.com))

## Hybrid Capture

- Hybridize DNA target with an RNA probe



Images courtesy of Digene Inc. ([www.qiagen.com](http://www.qiagen.com))

## Hybrid Capture

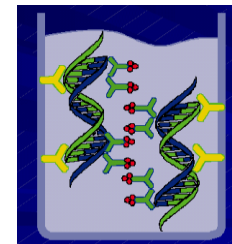
- Capture RNA:DNA hybrids onto a solid phase using anti-RNA:DNA duplex antibody



Images courtesy of Digene Inc. ([www.qiagen.com](http://www.qiagen.com))

## Hybrid Capture

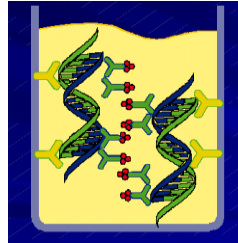
- React captured duplexes with multiple antibody conjugates



Images courtesy of Digene Inc. ([www.qiagen.com](http://www.qiagen.com))

## Hybrid Capture

- Detect with chemiluminescent substrate



Images courtesy of Digene Inc.  
(www.qiagen.com)

## Digene HPV HC2 Performance Data

Comparison of hc2 High-Risk HPV DNA Test Versus Consensus Histology  
ASC-US Referral Pap Population  
Kaiser Study  
PreservCyt Solution Specimens

	CIN 2-3 or cancer at the time of colposcopy			
		+	-	Total
hc2 High-Risk HPV DNA Test	+	66	317	383
	-	5	497	502
Total		71	814	885

Sensitivity (TP/(TP+FN)) = 93.0% (66/71)

95% CI = 84.3 to 97.7

Specificity (TN/(TN+FP)) = 61.1% (497/814)

95% CI = 57.7 to 64.4

Disease Prevalence = 8.0% (71/885)

Assay Positive Predictive Value = 17.2% (66/383)

Assay Negative Predictive Value = 99.0% (497/502)

Source: Digene HPV HC2 assay package insert

## Digene HPV HC2 Performance Data

Kaiser Study Data  
hc2 High-Risk HPV DNA Test Performance versus Consensus Histology Results (CIN 2-3)  
Age-Specific Characteristics

	Age < 30	Age 30 - 39	Age >39
<b>N</b>	287	233	365
<b>Prevalence of Disease (%)</b>	12.2	11.2	2.7
<b>Sensitivity (%)</b>	100.00 (35/35)	88.46 (23/26)	80.00 (8/10)
<b>95% Confidence Interval</b>	90.0-100	69.9-97.6	44.4-97.5
<b>Specificity (%)</b>	31.4 (79/252)	66.2 (137/207)	79.15 (281/355)
<b>95% Confidence Interval</b>	25.7-37.5	59.3-72.6	74.6-83.3
<b>Negative Predictive Value (%)</b>	100 (79/79)	97.86 (137/140)	99.29 (281/283)
<b>Positive Predictive Value (%)</b>	16.83 (35/208)	24.73 (23/93)	9.76 (8/82)

Source: Digene HPV HC2 assay package insert

## Third Wave Technologies Cervista

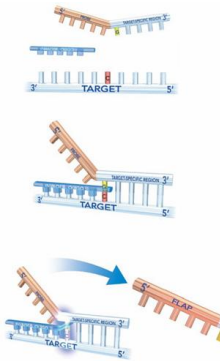
- To screen patients with atypical squamous cells of undetermined significance (ASC-US) cervical cytology results to determine the need for referral to colposcopy.

- Used adjunctively with cervical cytology to screen women 30 years and older to assess the presence or absence of high-risk HPV types.

## Third Wave Technologies Cervista

- Based on Invader Biplex technology
- Detects 14 high risk HPV strains
  - 16/18/31/33/35/39/45/51/52/56/58/59/68
- Specimen types
  - Liquid Pap Collection Media
    - Cytoc ThinPrep (FDA approved)
    - TriPath (BD) SurePath
- Two different tests
  - Cervista HPV HR
  - Cervista HPV16/18 Test
- Includes a positive internal control

## Invader Technology

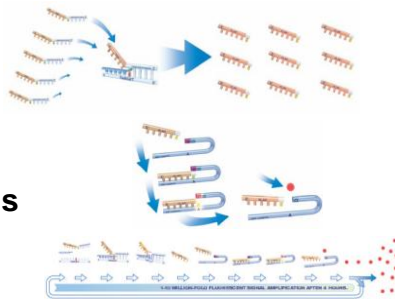


Courtesy Third Wave Technologies In.

## Invader Technology

Primary Rxn

Simultaneous  
2° reaction



Courtesy Third Wave Technologies In.

## Cervista HPV HR

**Table 11:** Clinical Performance Summary of the Cervista™ HPV HR Test as Compared to Colposcopy/Central Histology Results (≥ CIN2) among Women with ASC-US Cytology

Sensitivity	92.6% (64/69)	95% CI: (84.1% - 96.9%)
Specificity	44.2% (559/1263)	95% CI: (41.5% - 46.9%)
PPV	8.3% (64/769)	95% CI: (7.6% - 8.9%)
NPV	99.1% (558/563)	95% CI: (98.1% - 99.6%)
Disease Prevalence	5.2% (69/1332)	

Note: Among women with ASC-US cytology, there were 1.1% (15 out 1347) Cervista™ HPV HR indeterminate results with 95% CI: 0.7% to 1.8%.

**Table 12:** Clinical Performance Summary of the Cervista™ HPV HR Test as Compared to Colposcopy/Central Histology Results (≥ CIN3) among Women with ASC-US Cytology

Sensitivity	100% (2/22)	95% CI: (85.1% - 100%)
Specificity	43% (563/1310)	95% CI: (40.3% - 45.7%)
PPV	2.9% (22/769)	95% CI: (2.4% - 3.0%)
NPV	100% (563/563)	95% CI: (99.4% - 100%)
Disease Prevalence	1.7% (22/1332)	

Note: Among women with ASC-US cytology, there were 1.1% (15 out 1347) Cervista™ HPV HR indeterminate results with 95% CI: 0.7% to 1.8%. CIN2 histology results (47) are considered negative for disease (≥ CIN3) in this table.

Source: Cervista HPV HR package insert

## Cervista HPV HR

Age: 16 to <21	Central Histology = CIN2		
	Negative	Positive	Total
Cervista™ HPV HR			
Positive	96	9	105
Negative	28	0	28
Total	124	9	133
Disease Prevalence:	6.8% (9/133)	95% CI	
Sensitivity:	100% (9/9)	70.1%	100.0%
Specificity:	22.6% (28/124)	16.1%	30.7%
PPV:	8.6% (9/105)	4.0%	15.70%
NPV:	100% (28/28)	87.7%	100.0%
Age: 21 to <30	Central Histology = CIN2		
	Negative	Positive	Total
Cervista™ HPV HR			
Positive	321	31	352
Negative	136	0	136
Total	457	31	488
Disease Prevalence:	6.4% (31/488)	95% CI	
Sensitivity:	100% (31/31)	89.0%	100.0%
Specificity:	29.8% (136/457)	25.8%	34.1%
PPV:	8.8% (31/352)	6.1%	12.27%
NPV:	100% (136/136)	97.3%	100.0%
Age: 30 to <39	Central Histology = CIN2		
	Negative	Positive	Total
Cervista™ HPV HR			
Positive	157	10	167
Negative	126	3	129
Total	283	13	296
Disease Prevalence:	4.4% (13/296)	95% CI	
Sensitivity:	76.9% (10/13)	49.7%	91.8%
Specificity:	44.5% (126/283)	38.8%	50.3%
PPV:	6.0% (10/167)	2.9%	10.74%
NPV:	97.7% (126/129)	92.4%	99.9%
Age: 39 or older	Central Histology = CIN2		
	Negative	Positive	Total
Cervista™ HPV HR			
Positive	131	14	145
Negative	268	2	270
Total	399	16	415
Disease Prevalence:	3.9% (16/415)	95% CI	
Sensitivity:	87.5% (14/16)	64.0%	96.5%
Specificity:	67.2% (268/399)	62.4%	71.6%
PPV:	9.7% (14/145)	5.4%	15.67%
NPV:	99.3% (268/270)	97.3%	99.9%

Source: Cervista HPV HR package insert

## Cervista HPV 16/18

Risks of ≥ CIN2 for Different Outcomes of Cervista™ HPV HR and Cervista™ HPV16/18 Tests  
Prevalence of ≥ CIN2: 5.3%

Cervista™ HPV HR Result	Cervista™ HPV 16/18 Result	Risk	95% CI		Likelihood Ratio		95% CI	
HPV HR Positive	HPV16 and/or 18 Positive	17.1% (44/257)	13.0%	22.2%	3.72	2.93	4.54	
	HPV16 & 18 Negative	4.0% (20/500)	2.6%	6.1%	0.75	0.51	1.06	
HPV HR Negative	HPV16/18 Negative and/or Positive	0.9% (5/555)	0.4%	2.1%	0.17	0.07	0.36	

Risks of ≥ CIN3 for Different Outcomes of Cervista™ HPV HR and Cervista™ HPV16/18 Tests  
Prevalence of ≥ CIN3: 1.7%

Cervista™ HPV HR Result	Cervista™ HPV 16/18 Result	Risk	95% CI		Likelihood Ratio		95% CI	
HPV HR Positive	HPV16 and/or 18 Positive	6.6% (17/257)	4.2%	10.3%	4.15	2.99	5.08	
	HPV16 & 18 Negative	1.0% (5/500)	0.4%	2.3%	0.59	0.26	1.14	
HPV HR Negative	HPV16/18 Negative and/or Positive	0.0% (0/555)	0.0%	0.7%	0.00	0.00	0.37	

Source: Cervista HPV HR package insert

## Performance of Cervista

Detection of High-Risk Papillomavirus DNA with Commercial Invader-Technology-Based Analyte-Specific Reagents following Automated Extraction of DNA from Cervical Brushings in ThinPrep Media<sup>†</sup>

Ted E. Schutzbank,\* Charlene Jarvis, Nicole Kahmann, Katherine Lopez, Marlea Weimer, and Aleta Yount

JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2007, p. 4067-4069

Schutzbank et al, J. Clin. Microbiol. 2007;4067-4069

## Performance of Cervista

TABLE 1. Comparison of TWT Invader and Digene HPV testing results

Digene result	No. of samples with indicated TWT result	
	Positive	Negative
Positive	37	15
Negative	1	34

Schutzbank et al, J. Clin. Microbiol. 2007;4067-4069

## Performance of Cervista

TABLE 2. Resolution of TWT Invader and Digene discordant results

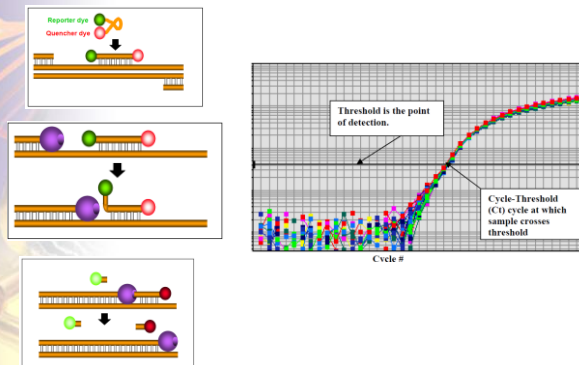
Sample no.	TWT Invader result	Digene result	HPV type
60Q	Negative	Positive	No HPV
13Q	Negative	Positive	No HPV
17Q	Negative	Positive	81
21Q	Negative	Positive	84
30Q	Negative	Positive	No HPV
49Q	Negative	Positive	30
57Q	Positive	Negative	51
62Q	Negative	Positive	70
65Q	Negative	Positive	42
76Q	Negative	Positive	No HPV
80Q	Negative	Positive	53
86Q	Negative	Positive	42

Schutzbank et al, J. Clin. Microbiol. 2007;4067-4069

## Roche cobas® HPV Test

- Qualitative in vitro test for the detection of Human Papillomavirus in patient specimens
- Utilizes amplification of target DNA by real-time Polymerase Chain Reaction (PCR)
- Detects 14 high-risk (HR) HPV types
  - 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68)
  - Differentiates types 16 and 18 from the remaining 12 HPV HR types.
- An additional primer pair and probe target the human  $\beta$ -globin gene (330 bp amplicon) to provide a process control.
- Sample type - Cervical specimens collected in PreservCyt solution using an endocervical brush/spatula

## TaqMan PCR



[http://www3.appliedbiosystems.com/cms/groups/mcb\\_marketing/documents/generaldocuments/cms\\_042484.pdf](http://www3.appliedbiosystems.com/cms/groups/mcb_marketing/documents/generaldocuments/cms_042484.pdf)

## Roche cobas® HPV Test

Performance	21-29 Years		30-39 Years		≥ 40 Years		
	N	606	417	640			
		≥ CR2					
Sensitivity (%)		88.4 (387/439)	100.0 (267/267)	88.7 (157/177)			
95% CI (%)		(79.5, 94.9)	(93.9, 100.0)	(81.7, 94.8)			
Specificity (%)		50.1 (292/740)	79.8 (292/740)	85.1 (332/740)			
95% CI (%)		(45.6, 54.6)	(69.6, 77.6)	(82.1, 87.7)			
NPV (%)		14.1 (26/268)	14.8 (26/712)	37.1 (97/183)			
95% CI (%)		(12.5, 15.9)	(13.8, 18.5)	(30.7, 33.9)			
PPV (%)		37.9 (292/733)	100.0 (267/268)	36.1 (332/737)			
95% CI (%)		(36.5, 39.1)	(97.4, 100.0)	(34.1, 39.5)			
≥ CR2 prevalence		4.5 (43/956)	4.8 (25/417)	2.3 (15/640)			
95% CI (%)		(3.4, 11.3)	(3.1, 7.3)	(1.4, 3.8)			
		≥ CR3					
Sensitivity (%)		95.8 (257/268)	100.0 (117/117)	72.7 (87/117)			
95% CI (%)		(79.6, 98.9)	(94.1, 100.0)	(63.4, 80.3)			
Specificity (%)		49.0 (298/740)	71.9 (292/740)	84.9 (354/740)			
95% CI (%)		(44.5, 53.4)	(67.4, 76.1)	(81.9, 87.5)			
NPV (%)		8.4 (25/289)	8.8 (11/125)	7.8 (8/103)			
95% CI (%)		(7.7, 9.5)	(7.3, 10.5)	(5.5, 11.2)			
PPV (%)		99.4 (267/233)	100.0 (267/268)	99.4 (324/737)			
95% CI (%)		(97.2, 99.9)	(97.5, 100.0)	(98.5, 99.8)			
≥ CR3 prevalence		4.7 (24/506)	2.6 (11/417)	1.7 (11/640)			
95% CI (%)		(3.2, 7.8)	(1.5, 4.7)	(1.0, 3.1)			

Roche cobas® HPV Test Package Insert

## Roche cobas® HPV Test Significance of Genotyping

Absolute Risk of  $\geq$  CIN2 and  $\geq$  CIN3 for Different cobas® HPV Test Results in the NILM Population ( $\geq$  30 Years)

cobas® HPV Test Result	$\geq$ CIN2	$\geq$ CIN3
<b>Unadjusted Estimates</b>		
HPV positive	6.3% (5.2, 7.5)	4.1% (3.5, 5.2)
HPV16 positive/18 positive	11.7% (8.9, 15.5)	8.9% (7.3, 10.5)
Other 12 HR positive	4.7% (3.7, 6.0)	2.9% (1.8, 3.5)
HPV negative	0.9% (0.4, 1.3)	0.3% (0.2, 0.4)
<b>Verification Bias Adjusted Estimates</b>		
HPV positive	6.1% (5.0, 7.3)	4.1% (3.5, 5.1)
HPV16 positive/18 positive	11.4% (8.3, 14.7)	8.7% (7.0, 10.4)
Other 12 HR positive	4.8% (3.5, 6.7)	2.8% (1.6, 3.3)
HPV negative	0.8% (0.3, 1.3)	0.3% (0.2, 0.7)

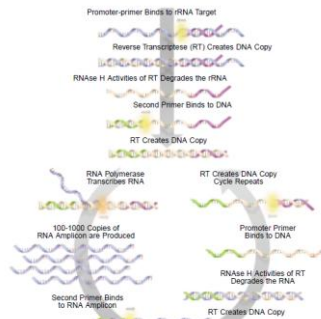
Note 1: HPV16 positive/18 positive include all subjects with either or both of these genotypes occurring with or without 12 Other HR HPV positive results.  
Note 2: 12 Other HR HPV positive include all subjects with positive results for 12 Other HR HPV genotypes with negative results for HPV16 and HPV18.

Roche cobas® HPV Test Package Insert

## APTIMA® HPV Assay

- The APTIMA HPV Assay is a target amplification nucleic acid probe test
  - Targets the HPV E6 and E7 transcripts
  - Detects 14 HPV high risk types
    - 16/18/31/33/35/39/45/51/52/56/58/59/66/68
    - Does not discriminate between the 14 high-risk types
  - Specimen collection
    - ThinPrep Pap Test vials containing PreservCyt Solution
    - APTIMA Cervical Specimen Collection and Transport Kit

## Transcription-Mediated Amplification



Courtesy, GenProbe Inc.

## APTIMA® HPV Assay

ASC-US to 2+ High Population: Performance of the APTIMA HPV Assay and an FDA-approved HPV DNA Test for Detection of  $\geq$ CIN2 and  $\geq$ CIN3

Performance	APTIMA HPV Assay Index		HPV DNA Test Index	
	Estimate	95% CI	Estimate	95% CI
<b>All Blisters</b>				
Sensitivity (%)	92.8	(78.4, 92.3)	92.8	(80.5, 98.8)
Specificity (%)	92.9	(89.6, 96.0)	92.9	(82.9, 98.5)
PPV (%)	21.1	(18.1, 22.0)	18.7	(17.0, 20.4)
NPV (%)	97.9	(96.9, 98.8)	97.9	(96.2, 98.8)
Prevalence (%)	1.7 (2.000)			
<b><math>\geq</math>CIN2</b>				
<b>Discorded Blisters*</b>				
Sensitivity (%)	92.3	(84.1, 97.4)	92.3	(83.8, 97.5)
Specificity (%)	92.2	(87.9, 96.4)	92.2	(81.0, 97.5)
PPV (%)	14.2	(12.7, 15.5)	12.1	(11.7, 14.2)
NPV (%)	98.3	(96.9, 99.0)	98.3	(97.9, 98.7)
Prevalence (%)	4.4 (2.000)			
<b>All Blisters</b>				
Sensitivity (%)	92.2	(77.5, 96.1)	92.2	(79.7, 97.5)
Specificity (%)	92.2	(87.5, 95.8)	92.2	(80.9, 98.4)
PPV (%)	8.4	(7.1, 10.4)	8.5	(7.4, 10.1)
NPV (%)	92.3	(90.3, 93.8)	92.3	(90.5, 93.8)
Prevalence (%)	4.4 (2.000)			
<b><math>\geq</math>CIN3</b>				
<b>Discorded Blisters*</b>				
Sensitivity (%)	92.1	(78.0, 98.1)	92.1	(82.9, 98.4)
Specificity (%)	92.1	(84.0, 98.2)	92.1	(80.4, 98.1)
PPV (%)	6.9	(5.6, 7.5)	6.4	(5.6, 7.2)
NPV (%)	98.8	(98.8, 100)	98.8	(98.8, 100)
Prevalence (%)	3.2 (2.000)			

APTIMA® HPV Assay Package Insert

## APTIMA® HPV Assay

ASC-US ≥ 21 Years Population: Performance of the APTIMA HPV Assay and an FDA-approved HPV DNA Test for Detection of ≥CIN2 by Age Group

Performance	APTIMA HPV Assay N=938		HPV DNA Test N=865	
	Estimate	(95% CI)	Estimate	(95% CI)
<b>21 to 29 Years</b>				
Sensitivity (%)	80.2 (55/67)	(60.2, 95.4)	84.9 (55/69)	(65.1, 98.3)
Specificity (%)	44.3 (159/354)	(39.8, 50.1)	35.5 (117/330)	(30.5, 40.8)
PPV (%)	32.2 (55/250)	(19.6, 24.2)	30.8 (56/289)	(19.0, 22.5)
NPV (%)	90.4 (159/165)	(83.5, 98.5)	97.5 (117/120)	(93.5, 99.4)
Prevalence (%)	14.7 (8/1415)		15.2 (23/389)	
<b>30 to 39 Years</b>				
Sensitivity (%)	80.0 (16/20)	(69.9, 97.2)	80.0 (16/20)	(58.4, 91.9)
Specificity (%)	68.2 (103/242)	(62.1, 73.7)	61.8 (132/219)	(55.1, 67.8)
PPV (%)	19.9 (18/95)	(14.7, 22.7)	16.0 (16/100)	(11.8, 19.6)
NPV (%)	98.8 (165/167)	(96.5, 99.8)	99.1 (135/139)	(94.1, 99.1)
Prevalence (%)	7.6 (23/292)		8.4 (23/279)	
<b>≥ 40 Years</b>				
Sensitivity (%)	80.0 (8/10)	(31.3, 83.2)	70.0 (7/10)	(37.7, 89.2)
Specificity (%)	62.9 (206/252)	(77.8, 87.1)	78.7 (191/227)	(74.0, 84.4)
PPV (%)	12.2 (8/69)	(5.8, 18.4)	13.2 (7/53)	(8.9, 18.7)
NPV (%)	98.1 (206/213)	(96.8, 99.4)	98.4 (181/184)	(96.8, 99.8)
Prevalence (%)	3.8 (19/262)		4.2 (19/237)	

APTIMA® HPV Assay Package Insert

## HPV In Situ Hybridization

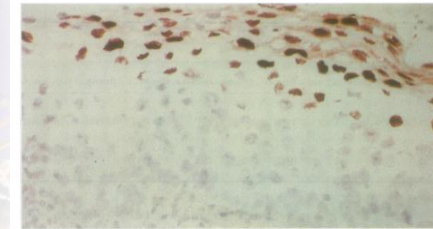


Figure 2 Type 1 pattern of red NISH signal confined to the upper half of the squamous epithelium.

Cooper et al. J. Clin Pathol. 1991; 44:400-405

## PCR Reverse Line Probe HPV Genotyping Assays

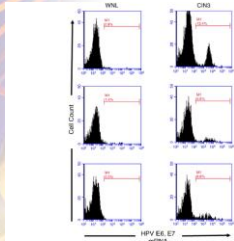
- Line probe assay
  - Based on the reverse hybridization principle
  - Innogenetics LiPA
    - Identifies 28 different HPV genotypes
      - High risk - 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82
      - Low risk - 6, 11, 40, 43, 44, 54, 70

## PCR Reverse Line Probe HPV Genotyping Assays

- Roche Molecular Diagnostics LINEAR ARRAY® HPV Genotyping Test
  - Detects 37 different HPV genotypes
    - 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73 (MM9), 81, 82 (MM4), 83 (MM7), 84 (MM8), IS39, and CP6108 (high-risk in bold)



## Flow Cytometric Analysis of E6/E7 Transcripts



Comparison of Flow Cytometry to HC2 assay

	Hybrid Capture 2	Flow Cytometry
Sensitivity	89%	84%
Specificity	78%	96%
PPV	43%	78%

G. Coquillard et al. / Gynecologic Oncology 120 (2011) 89–93

## Flow Cytometric Analysis of E6/E7 Transcripts

### Stability of RNA transcripts over time

**Table 1**  
Liquid-based cervical cytology specimen stability as determined by signal to noise ratio (SNR) degradation over time at 25 °C storage using positive and negative control cells preserved in SurePath liquid.

Time	Mean fluorescence intensity (MFI)	
	Positive control cells	Negative control cells
Day 0	11.4	0.7
Day 7	13.1	0.8
Day 14	10.9	0.7
Day 21	12	0.9
Month 1	14.2	1
Month 3	9.8	1.6

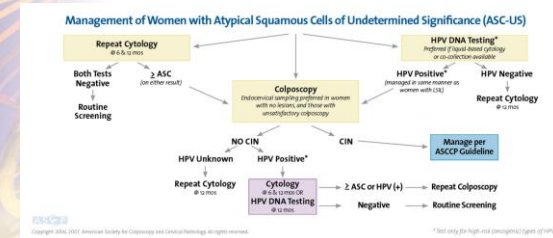
G. Coquillard et al. / Gynecologic Oncology 120 (2011) 89–93

## Cervical Cancer Screening Guidelines

- Cervical cancer screening should begin at the age of 21.
- Most women under the age of 30 should be screened every two years.
- Women age 30 and older who have had three consecutive normal Pap tests can be screened every three years.
- Women with certain risk factors may need to be screened more frequently. These risk factors include HIV positivity; immunosuppression; DES exposure in utero; or history of treatment for cervical intraepithelial neoplasia (CIN) 2, CIN 3, or cervical cancer.
- Women who have had a total hysterectomy (removal of the cervix and uterus) for reasons other than cancer can stop being screened for cervical cancer unless they have a history of high-grade CIN.

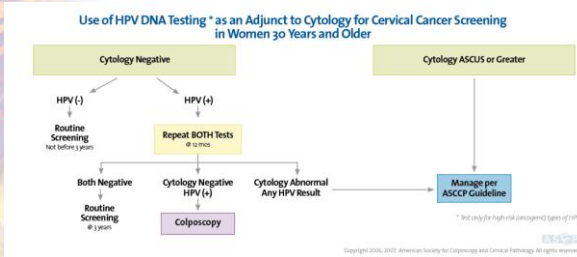
Source: www.acog.org

## Utility of HPV DNA Testing: American Society for Colposcopy and Cervical Pathology (ASCCP) Guidelines



Source: <http://www.asccp.org/ConsensusGuidelines/AbnormalCervicalCancerScreeningTests/tabid/5958/Default.aspx>

## Utility of HPV DNA Testing: ASCCP Guidelines



Source: [http://www.asccp.org/Portals/9/docs/pdfs/Consensus%20Guidelines/algorithms\\_cyto\\_07.pdf](http://www.asccp.org/Portals/9/docs/pdfs/Consensus%20Guidelines/algorithms_cyto_07.pdf)

## Rationale for Genotyping Frequency of HPV Genotypes in Cervical Cancer

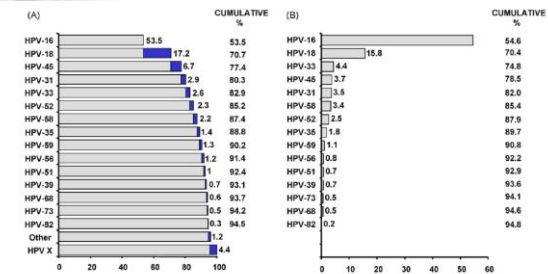


Fig. 1. Percentages of cervical cancer cases attributed to the most frequent HPV types in all world regions combined, as estimated from: (A) the IARC pooled analysis of 3085 cases (adapted from [8]), and (B) meta-analysis of more than 14,500 cases (adapted from [11]).

Clifford, K et al., Vaccine 2006, 24: S26-S34. Elsevier Copyright © 2006 Elsevier Ltd. All rights reserved.

## Rationale for Genotyping

- Findings from the ASCUS/LSIL Triage Study (ALTS)
  - Detection of HPV-16 or -18 at entry into the study was associated with a very high risk of CIN3
  - Types other than 16 had a collective risk of CIN3 that was ~one-fifth of that for HPV-16 (7.9% vs. 39.1%).
  - multiple infections with non-HPV-16 carcinogenic types significantly increased the risk of CIN3, compared with infection with single-type non-HPV-16 carcinogenic infections

Wheeler et al. JID 2006;194, 1291 - 199

Thank you for your kind attention

Questions?



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