

HPV Genotyping Solutions by QIAGEN

digene HPV Genotyping RH Test

1

↑The management of cervical precancer is same for any oncogenic HPV types. Why genotype HPV then?

↑The severity of progression differs for different HPV types, for instance, type 16 that causes ~50% ca, has a faster progression rate than any other.

↑In practice, the management may be different: The CIN2 caused by type 16 is much less likely to regress, and hence needs to be treated whilst by other types, may be followed up for some time at least.

Variations in the 2-yr incidence of CIN 2 and CIN 3++ in tx arms	2-year cumulative incidence		
	Conservative management	HPV Triage	Immediate colposcopy
CIN 3 ++	10.9%	10.3%	10.9%
CIN 2	5.8%	7.8%	9.9%

The relative differences in incidence of CIN2, by study arm, among women who tested HPV-16 positive at baseline were less pronounced than women who tested positive for other high-risk-HPV genotypes.

Philip E Castle, ALTS Study results. Obstet Gynaecol 2009; 113:18-25

2

To discriminate between transient and persistent infection

To identify a persistent infection one needs at least two consecutive tests with the ability for genotyping, because of the possibility of a new infection with another high risk type during the follow up period. (11% in Tuebingen)

Persistent high risk type HPV infection is a necessary prerequisite for the development, maintenance and progression of HSIL and for subsequent risk for invasive cancer

HPV DNA IN THE TISSUE OF CERVICAL CARCINOMA, IN SENTINEL AND PELVIC LYMPH NODES - CORRELATION WITH HISTOPATHOLOGICAL FINDINGS

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Background: Metastatic involvement of pelvic lymph nodes is the most important prognostic parameter in early-stages cervical cancer. Still, almost 20% of patients with negative nodes experience recurrence. Prognostic significance of the presence of DNA of the most common high risk (HR) genotypes HPV 16/18 in histopathology-negative pelvic nodes is considered as subclinical metastatic spread. Prognostic significance of such findings was shown in a few studies.

Objective: The main objective of the study was to evaluate the presence of HR HPV DNA including 13 genotypes in pelvic SLN, other pelvic nodes and in the tumour, and to correlate the findings with histopathological results.

Material and methods: Enrolled were patients with early-stage cervical cancer referred for surgical treatment including pelvic lymphadenectomy. During the surgery, samples for HR HPV assessment were taken. All samples were evaluated for the presence of HR HPV and genotyped for the main 13 genotypes.

Results: The study included 49 patients who underwent radical. Overall 91.8% patients had HPV in their primary tumours and 49.9% patients in SLN or other pelvic nodes. Among 10 detected genotypes, HPV 16 was the most represented. All metastatic lymph nodes were HPVpositive, and in all cases showed an absolute consistency with the primary tumour in terms of HPV genotypes.

Conclusion: The presence of HR HPV DNA in pelvic lymph nodes might be an early sign of subclinical metastatic spread. The evidence of HPV in SLN had a 100% positive predictive value for metastatic involvement of pelvic nodes.



Leverage the Gold Standard: Hybrid Capture

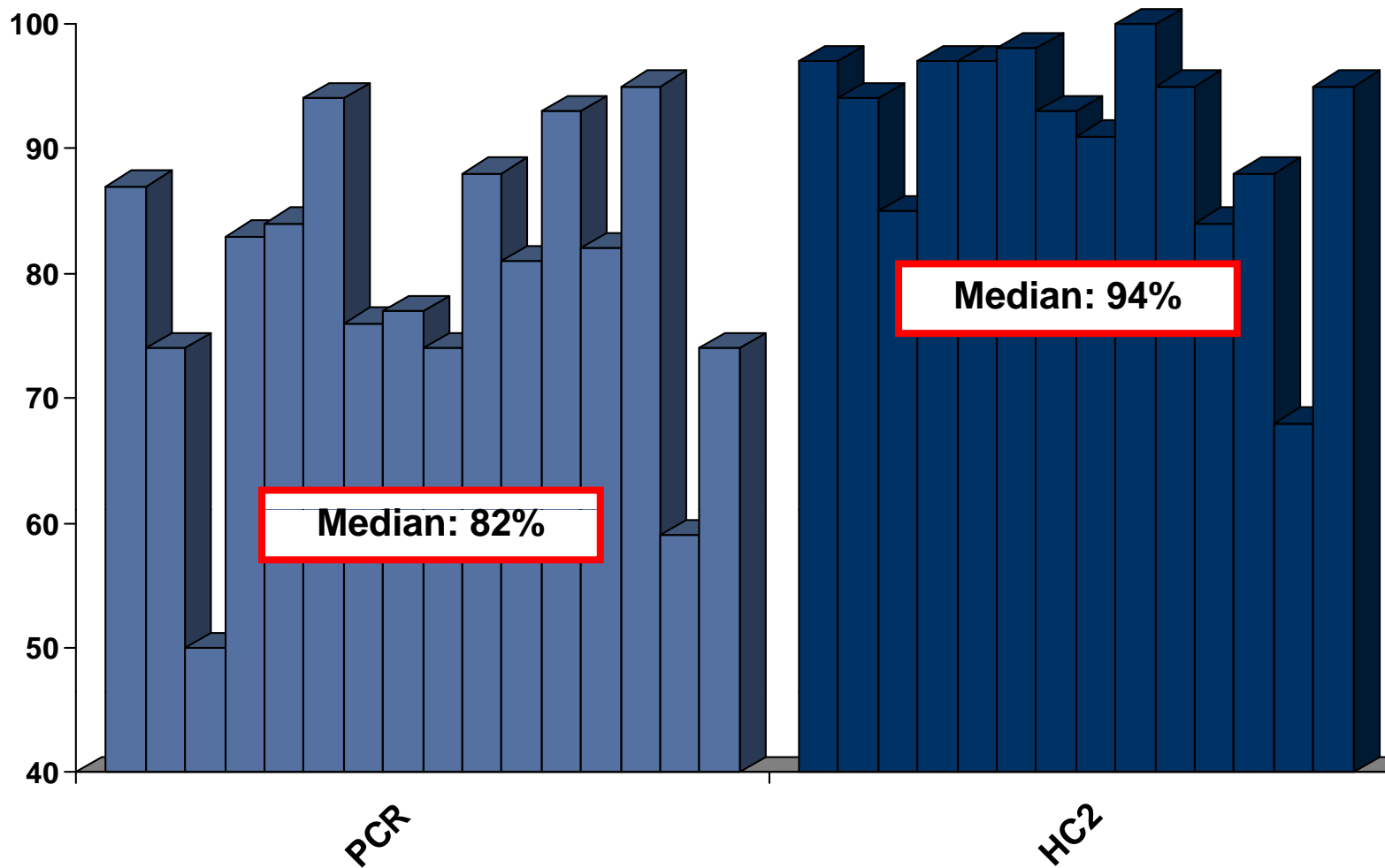
Author	Year	No. of Women
Lorincz	92	2,627
Cuzick	99	1,703
Bozzetti	00	977
Ratnam	00	2,098
Schiffman	00	8,554
Wright	00	1,365
Zielinski	01	278
Solomon	01	3,488
Belinson	01	1,997
Clavel	01	5,671
Pretorius	02	845
Kulasingam	02	4,075
Salmeron	03	7,732
Sherman	03	20,810

Author	Year	No. of Women
Petry	03	7,592
Cuzick	03	10,358
Kulmala	04	1,511
Castle	05	5,060 (ALTS 20k+)
Schiffman	05	10,000
Schiffman	05	3,363
Soderlund	05	239
Fetterman	05	123,909
Sarian	05	4,195
Bigras	05	13,842
Sankar.	05	36,938
Ronco	06	16,706
Kjaer / Iftner	06	10,234
Franco	06	9,667

315,000+ women
Hundreds of thousands of samples

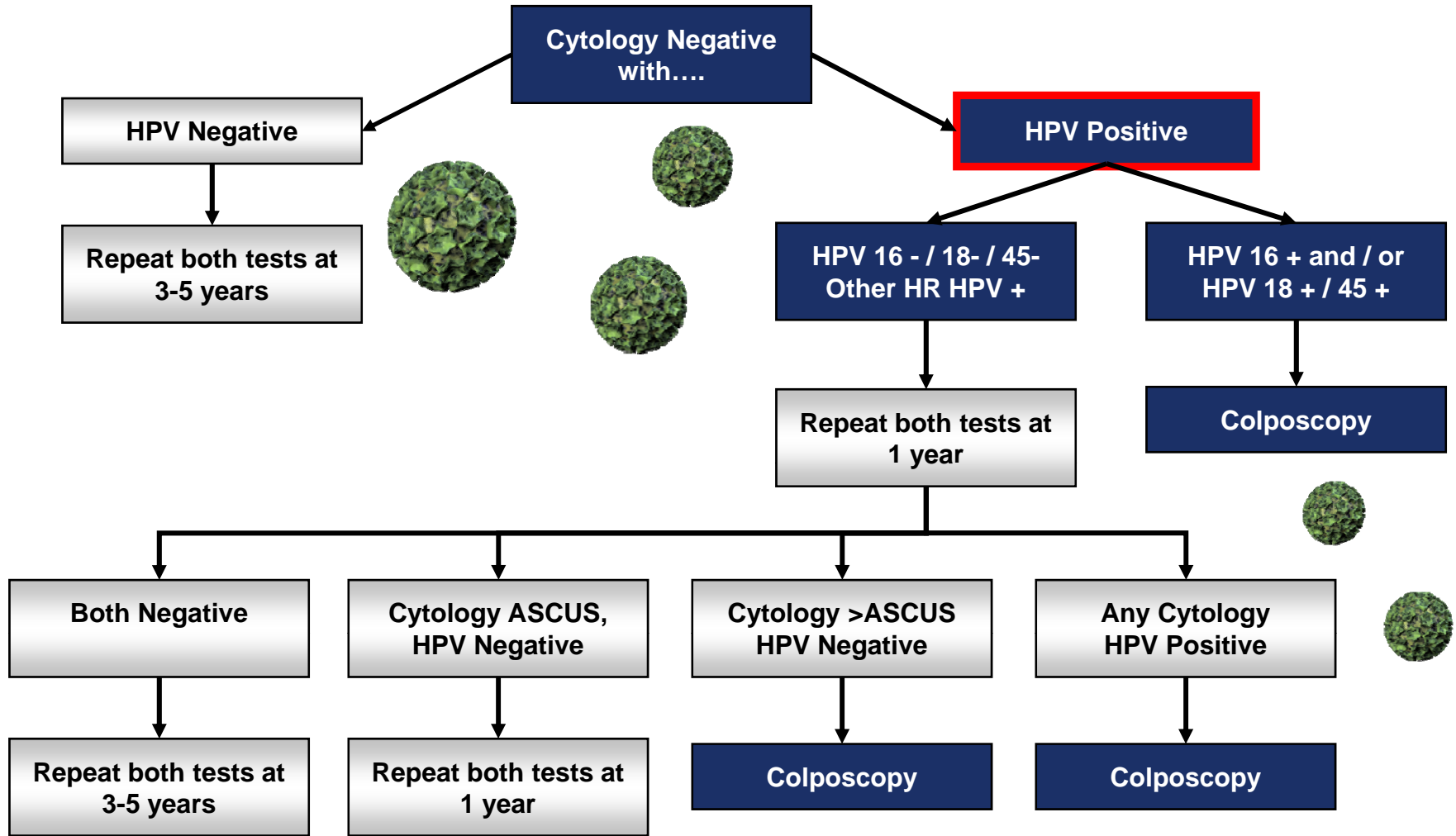


Clinical Sensitivity Comparison: HC2 vs. PCR

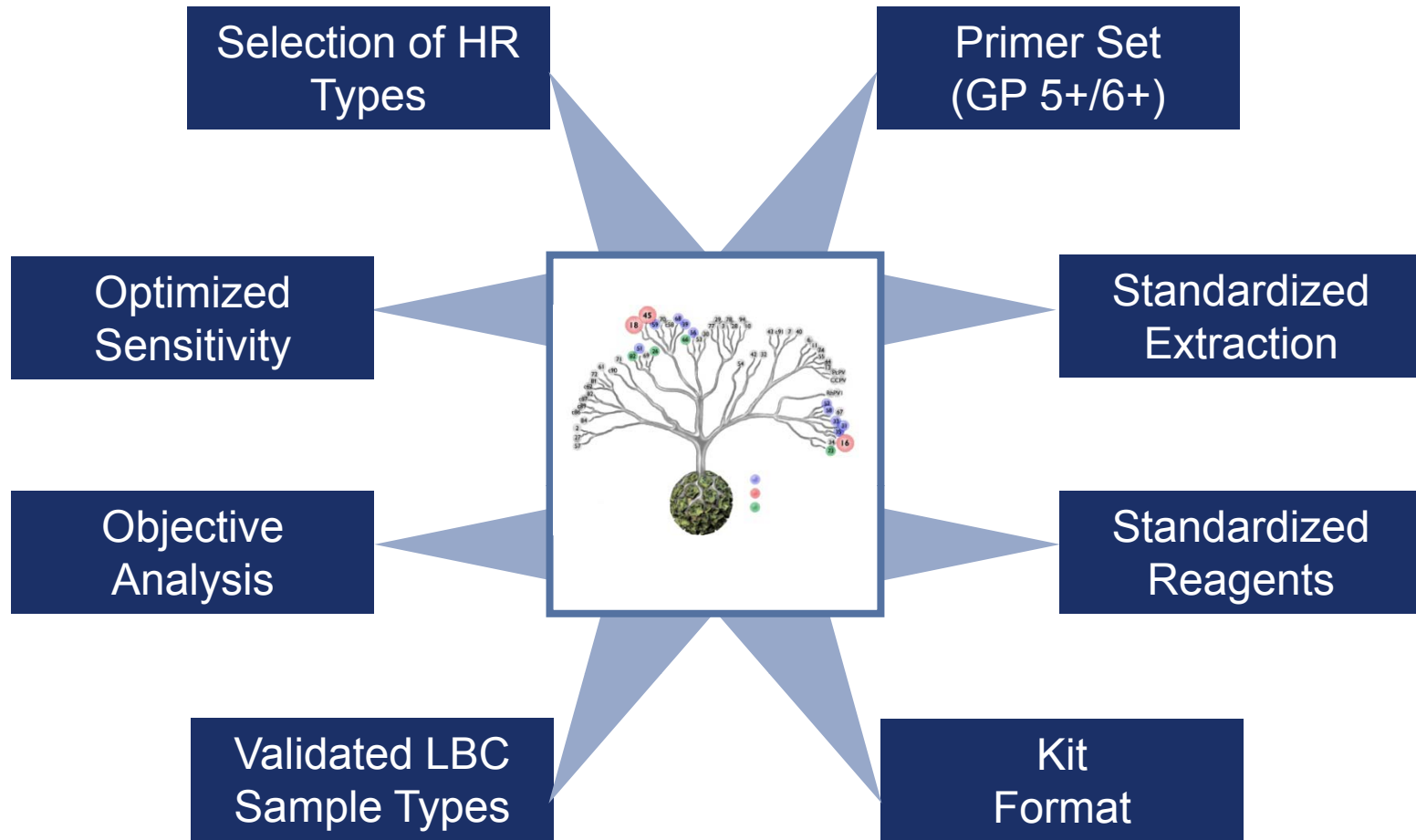


HC2 shows consistent clinical sensitivity – important for a screening assay

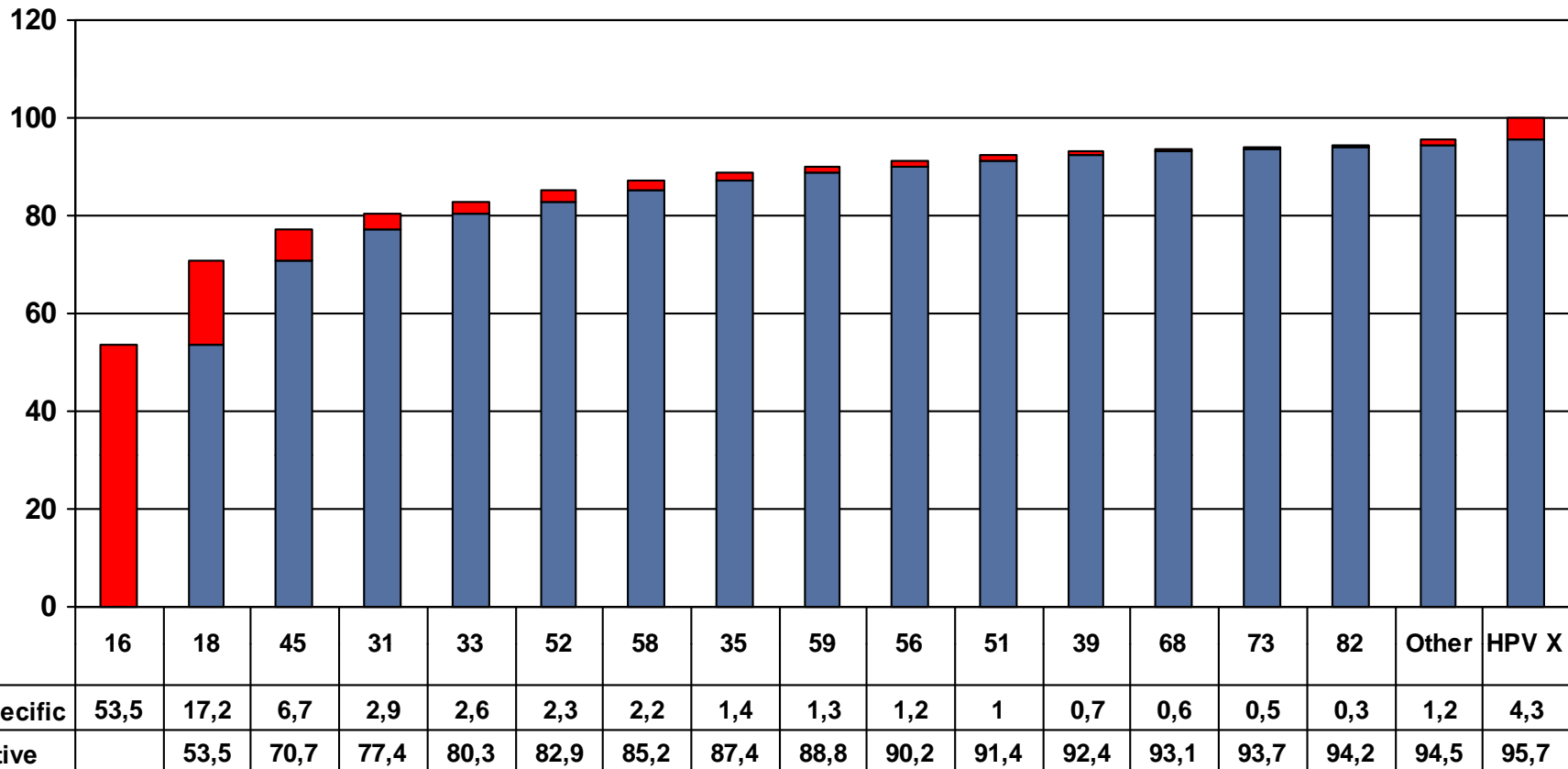
Genotyping – Proposed Algorithm



The Qiagen Approach – Assay Design



Cervical Cancer by Type Frequency



GT Assay HR: 16, 18, 45, 31, 33, 52, 58, 35, 59, 56, 51, 39, 68, 73, 82 also 53, 66, 26

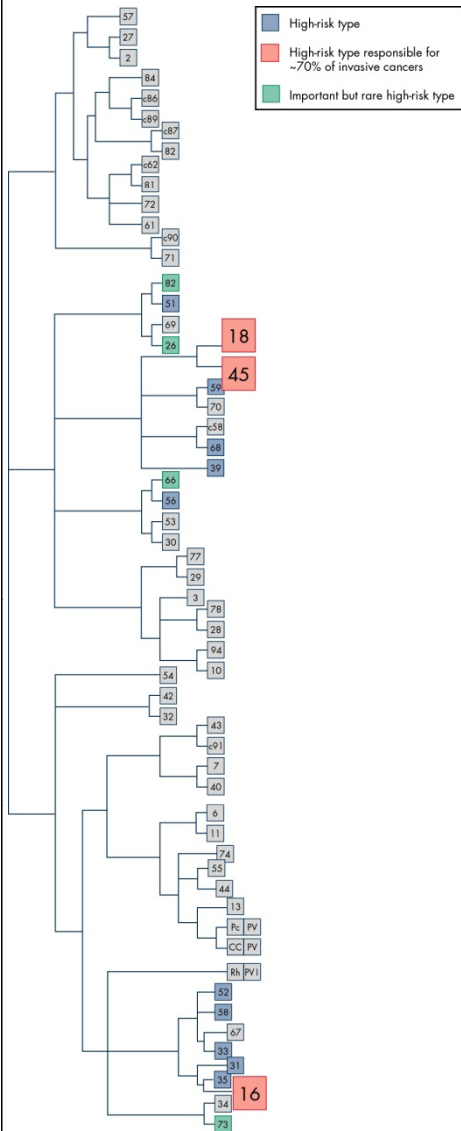
Munoz et al, Int J Cancer, 2004;111 (2): 571-9 Against which HPV Types should we vaccinate and screen? The international perspective



Genotyping – GP5+/6+ Primer Selection

- Primer set is well-published, understood and correlates well with HC2
- GP5+/6+ validated in large scale clinical trials

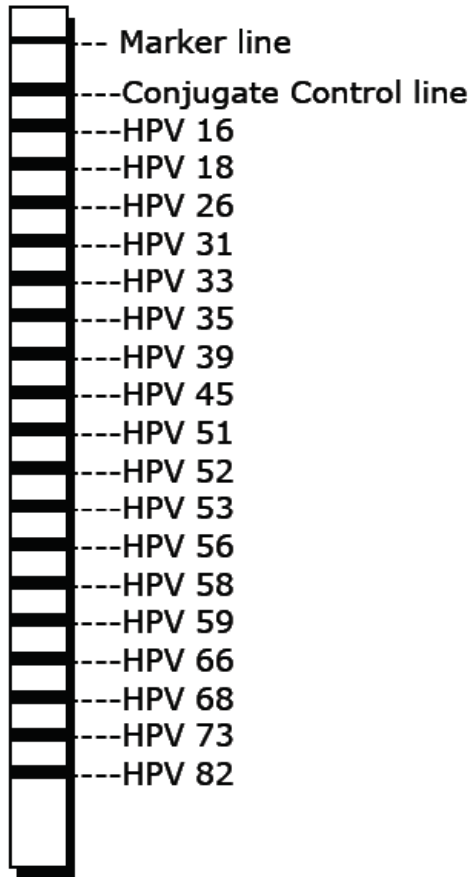
Investigator	Country	Year	Study Size	Reference
Kjaer S	Denmark	2002	10,758	BJM 2002 Sept 14; 325 (7364)
Johnson T	Denmark	2003	1,283	APMIS 2003 Mar;111(3):398-404
Ortiz M	Spain	2006	1,889	J Clin Micro Apr;44(4):1428-34
Cuschieri KS	Scotland	2004	3,444	J Clin Path Jan, 57(1) 62-72
Clifford GM (IARC)	France	2005	15,613	Lancet 2005, Sept 17-23, 366 (9490)
Forslund O	Sweden	2002	6,123	J Med Vir 2002 Apr;66(4):535-41
Ferruccio C	Chile	2004	1,038	Cancer Epid Biomarkers Prev. 2004 Dec;13(12):2271-6
Ronco	Italy	2005	1,013	Eur J Cancer 2005 Jan;41(2):297-305
Thomas JO	Nigeria	2004	932	Br J Cancer 2004 Feb 9;90(3):638-45.
Molano M	Colombia	2002	1,859	Br J Cancer 2002 Jul 29;87(3):324-33
Castellsaque X	Spain	2006	1,881	J Natl Cancer Inst 2006 Mar 1;98 (5):303-15
Hesselink AT	Netherlands	2006	8,132	J Clin Micro 2006 Oct;44(10):3680-5
Total Study Size			53,965	



Clinical management tool – requires a clinically validated primer:

- ❑ Risk stratification of women with types 16, 18, and 45 to determine the best course of action for optimal patient care and management
- ❑ Risk stratification of women with repeat positive samples of the same HPV type (persistence)
- ❑ Follow up on persistence post-LEEP (recurrence of CIN3+)

> Require GP5+/GP6+ Genotyping Assay



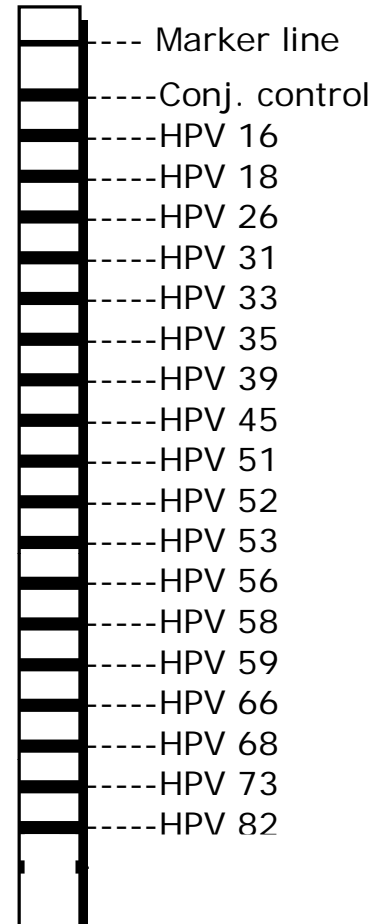
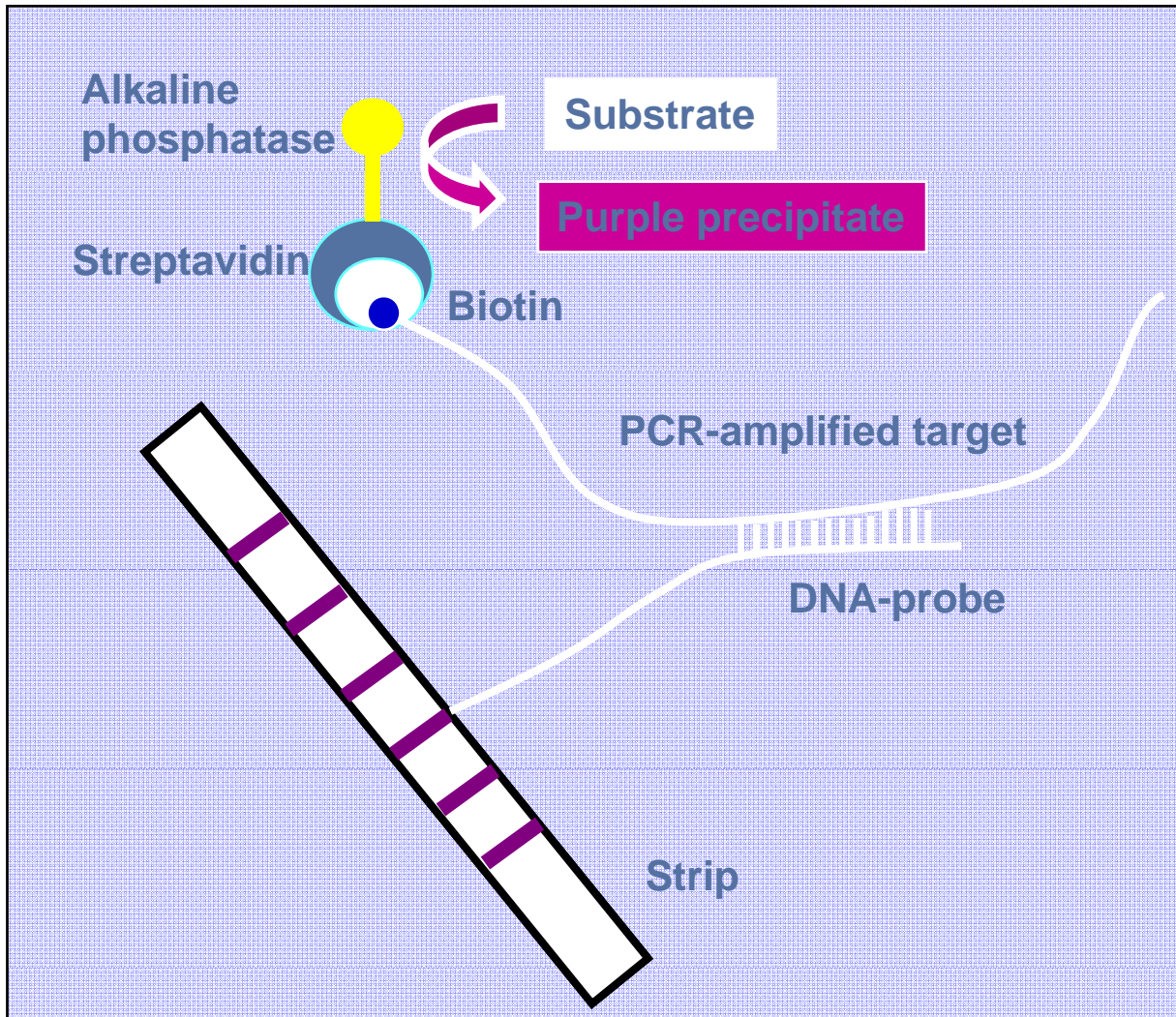
- PCR – based, using clinically validated GP5+/6+ Primer Set.
- Type specific follow-up of *digene* High-Risk HPV HC2 positive samples.
- Easy and reliable, for use with cervical specimen collected in STM™ (QIAGEN) or PreservCyt™ (Cytoc/Hologic).

Three step technology:

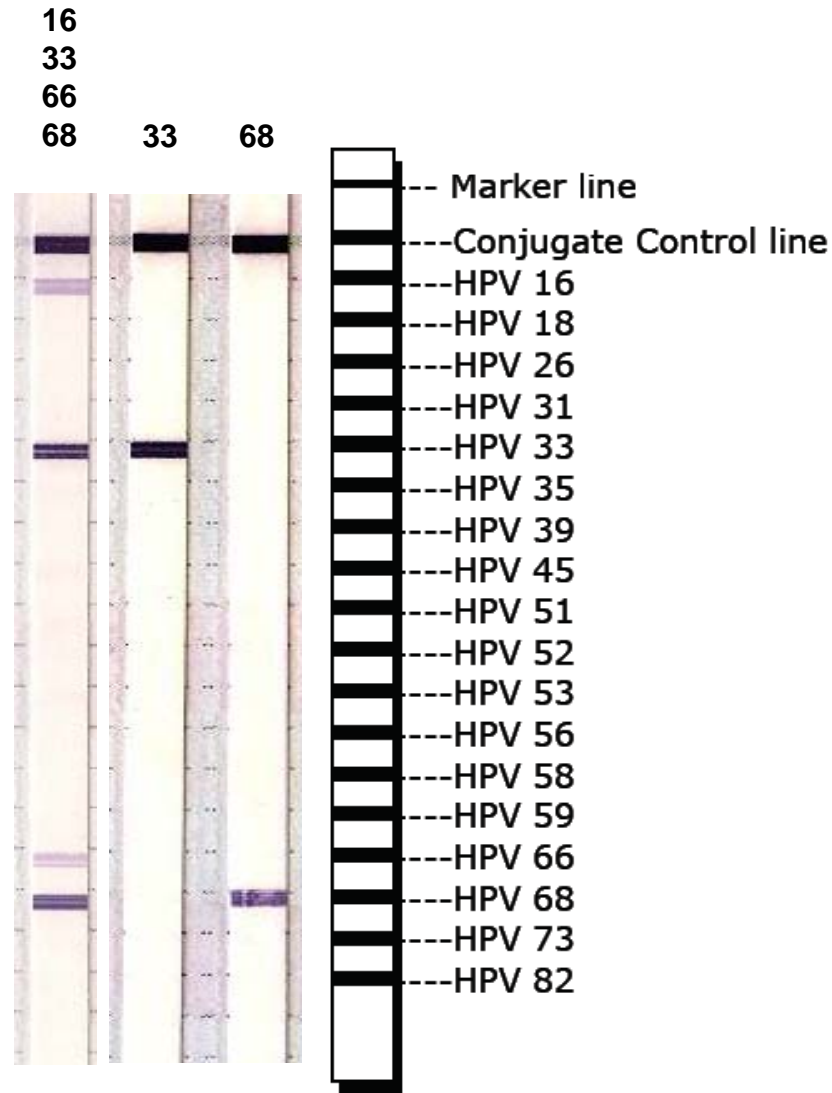
- DNA isolation
 - Consensus primer-based GP5+/6+ PCR
 - Targeted sequences within the highly conservative L1 region
 - Reverse Hybridization
 - One membrane strip coated with multiple probes allows the simultaneous detection of 18 HR HPV genotypes
 - Detection of 18 *high risk* or *probably high risk* types:¹
- 16, 18, 26, 31, 33, 35, 39, 45, 51,
52, 53, 56, 58, 59, 66, 68, 73, 82

¹ Munoz et al. 2003; *New Engl. J. Med.* 348:518-27;

Reverse Hybridization Assay – Principle



Visual interpretation of the genotyping results (using a data report sheet):



digene HPV Genotyping RH Test (RUO)

Detection Kit (20 tests)

- Reagents for the detection of GP5+/6+ amplimers of high risk HPV subtypes (18 types)

Primer Kit (20 tests)

- GP5+/6+ primers

Available November 2008



Manual protocol and protocol for AutoLipa / ProfiBlot validated and available

digene HPV Genotyping RH Test (CE)

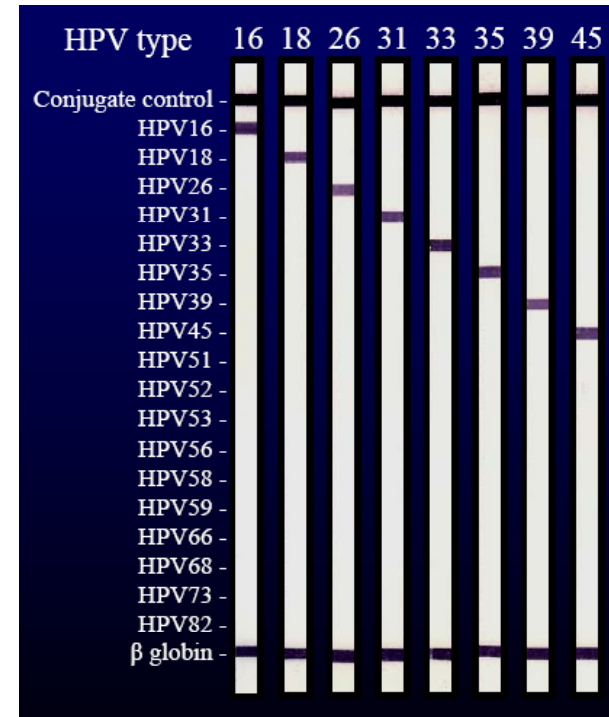
Detection Kit (20 tests)

- Reagents for the detection of GP5+/6+ amplimers of high risk HPV subtypes (18 types)

Amplification Kit (20 tests)

- Mastermix (incl. GP5+/6+ primers, β -Globin primer, enzymes)
- Mg solution
- Positive PCR Control

Available July 2009



Manual protocol and protocol for AutoLipa / ProfiBlot validated and available