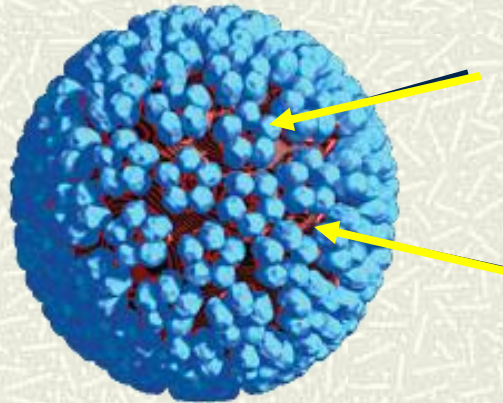


# Self sampling and HPV



- **Alexandros Daponte MD;Doctorate (LMU Munic);FCOG (SA)**

# Summary of European and North American studies

	<b>Sensitivity</b>	<b>Specificity</b>
<b>HPV</b>	96%	92%
<b>CYTOLOGY</b>	53%	97%

## *HUMAN PAPILLOMAVIRUS (HPV) AND CANCER*

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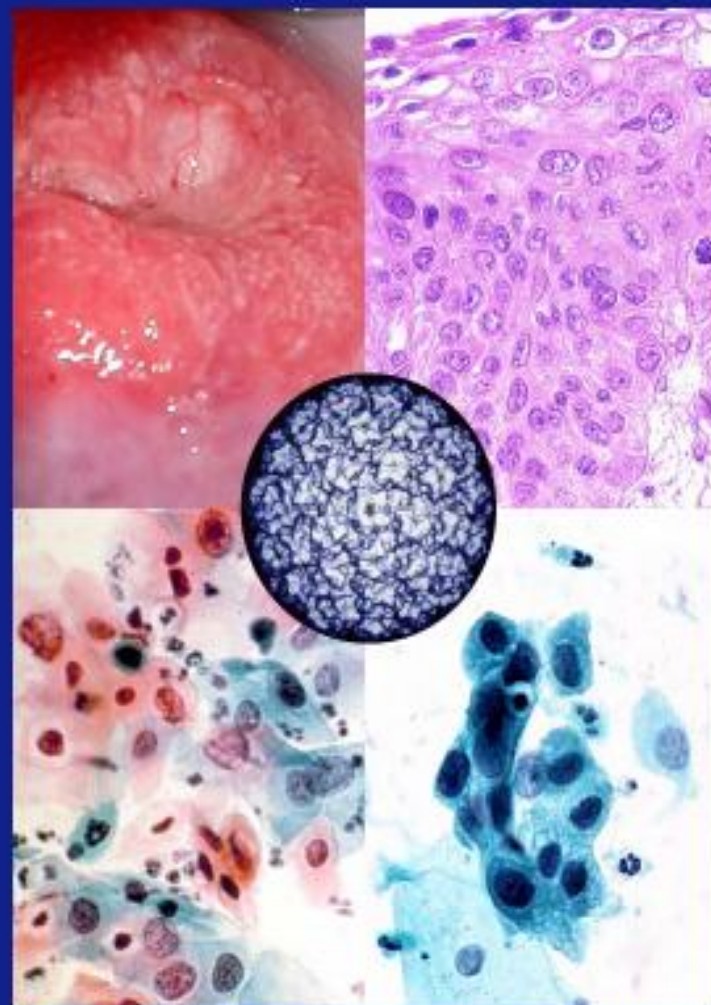
- ❖ HPV is now well established as the necessary cause of cervical cancer
  - ❖ increasing trend to use HPV-DNA testing for screening, triage and follow-up purposes.
-

# 2<sup>nd</sup> edition: European Guidelines on Quality Assurance in Cervical Cancer Screening

Luxembourg: Office for Official Publications  
of the European Communities (2008)

Editors

Arbyn M, Anttila A, Jordan J, Ronco G, Schenck U,  
Segnan N, Wiener H, Herbert A, von Karsa L  
(IARC, Lyon)



European guidelines for quality assurance  
in cervical cancer screening *Second Edition*



European Commission

# European Council recommendation

(2 December 2003)

---

- # Screening only offered in organised programmes
  - Monitoring & QA at all levels: invitation, participation, screen test, follow-up of screen+, treatment, after-care, registration, data-linkage (privacy!)
- # Screening in accordance with evidence-based guidelines

# Primary screening of HPV? (2008)

- **Not (yet) in mass screening programmes**
- **Accepted only in quality-controlled pilot programmes**
- **Further evidence of efficacy and effectiveness needed:**
  - **less CIN3 in 2<sup>nd</sup> screening round of RCTs in HPV- compared to cyto- at baseline**
  - **accepted as surrogate for reduced incidence of cancer**

# Supplements to the 2<sup>nd</sup> edition

---

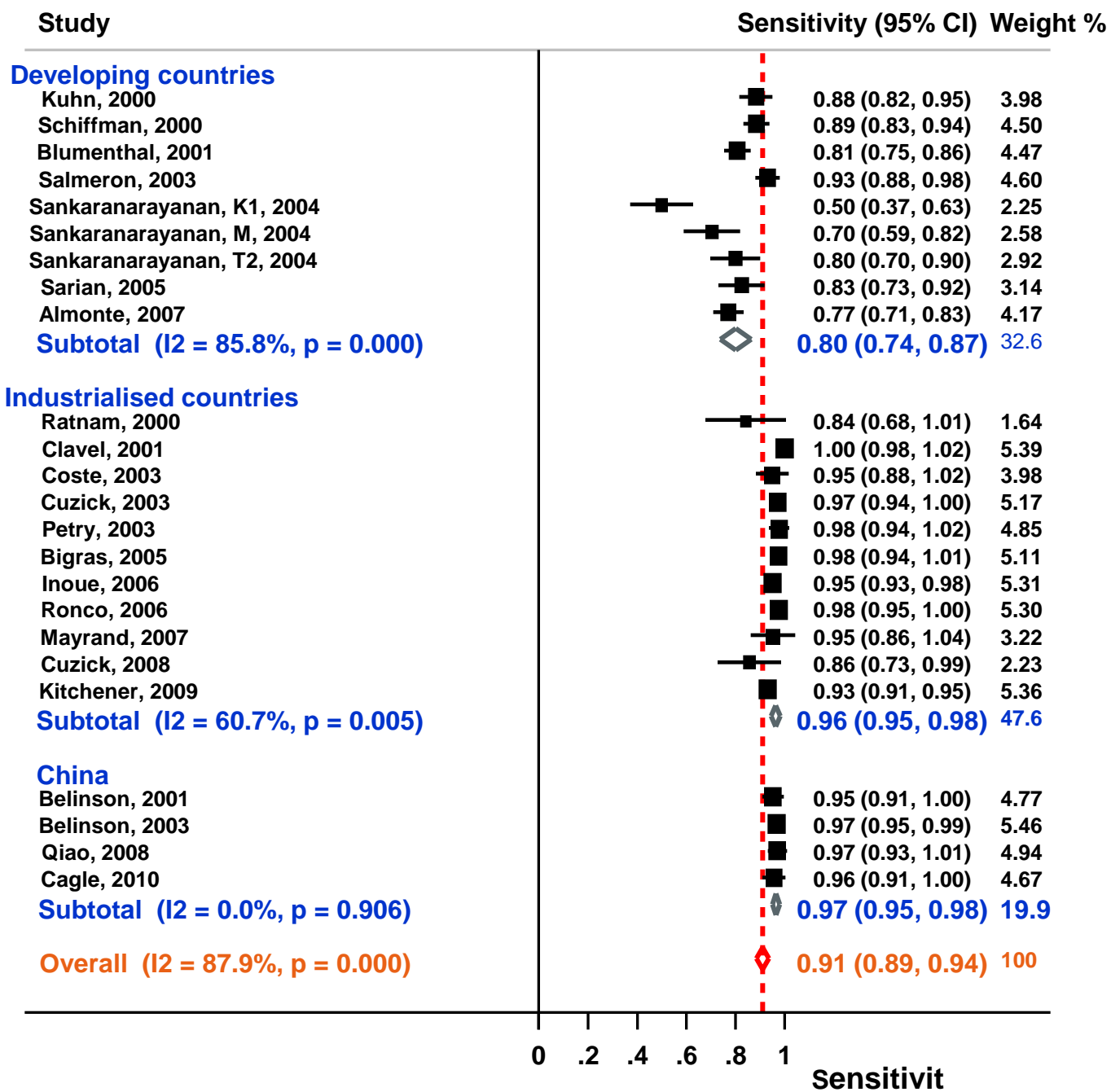
## ➔ # HPV testing in:

- Triage of minor cytology
- Follow-up after treatment
- Primary screening
- Quality control of cervical cytology

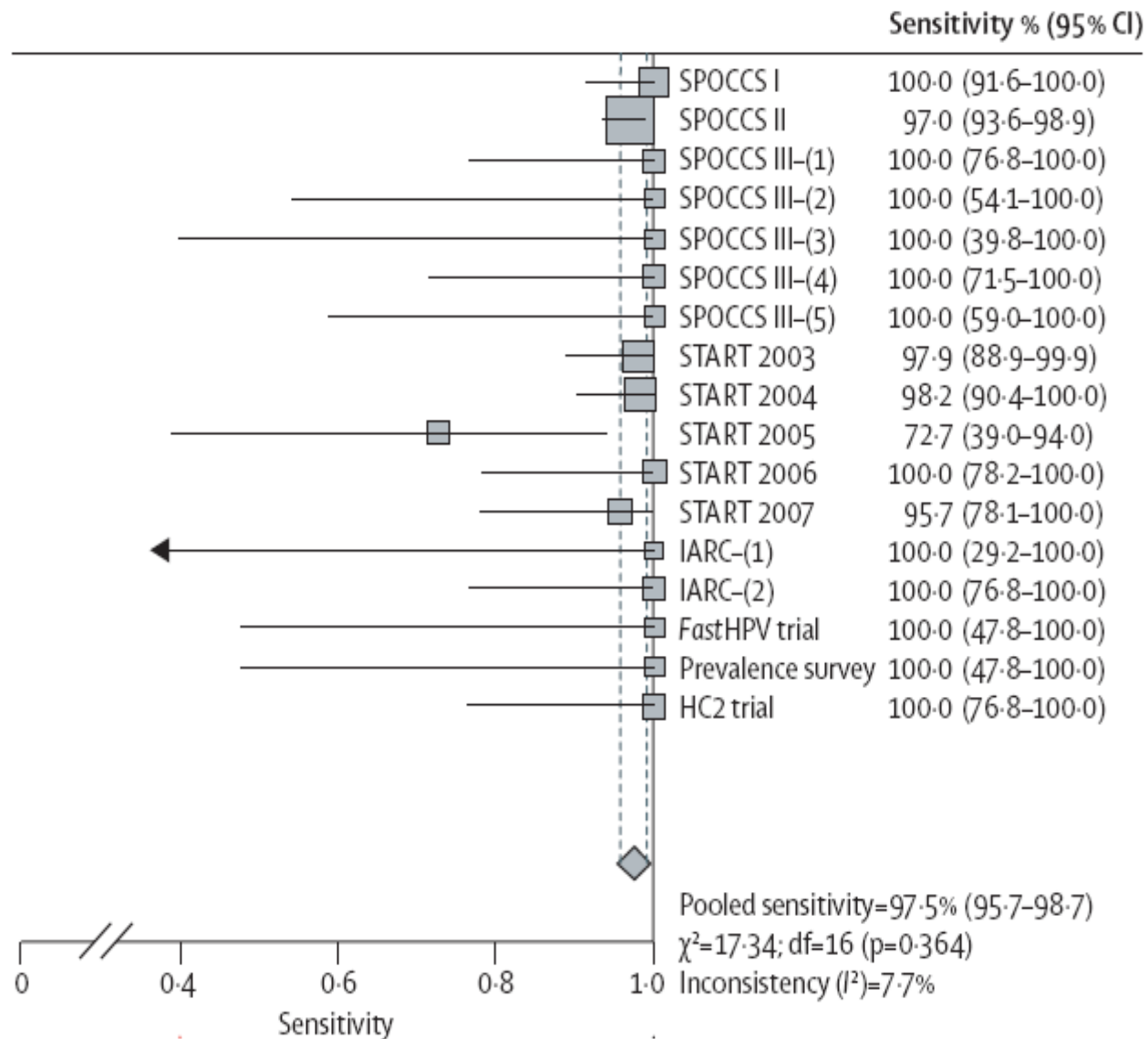
## # HPV vaccination

**HPV testing in 1ary screening:  
Accuracy to detect hgCIN  
(cross-sectional studies)**

# Sensitivity of HC2 to detect CIN2+ in 1ary screening



# Sensitivity of HC2 for CIN3+ (China)



# Accuracy of HC2 to detect high-grade CIN

## 1. Absolute sensitivity:

- CIN2+: 91% (95% CI: 88.6-93.6%) [24 studies]
- CIN3+: 98% (95% CI: 96.4-98.9%) [7 studies]

## 2. Specificity:

- CIN2+: 88% (95% CI: 86.7-90.2%) [23 studies]

## 3. Relative sensitivity vs cytology

- 1.23 (1.14-1.33) if ASCUS+; 1.38(1.25-1.52) if LSIL+

## 4. Relative specificity vs cytology

- 0.97(0.96-0.98) if ASCUS+; 0.91(0.90-0.92) if LSIL+

# Randomised trials

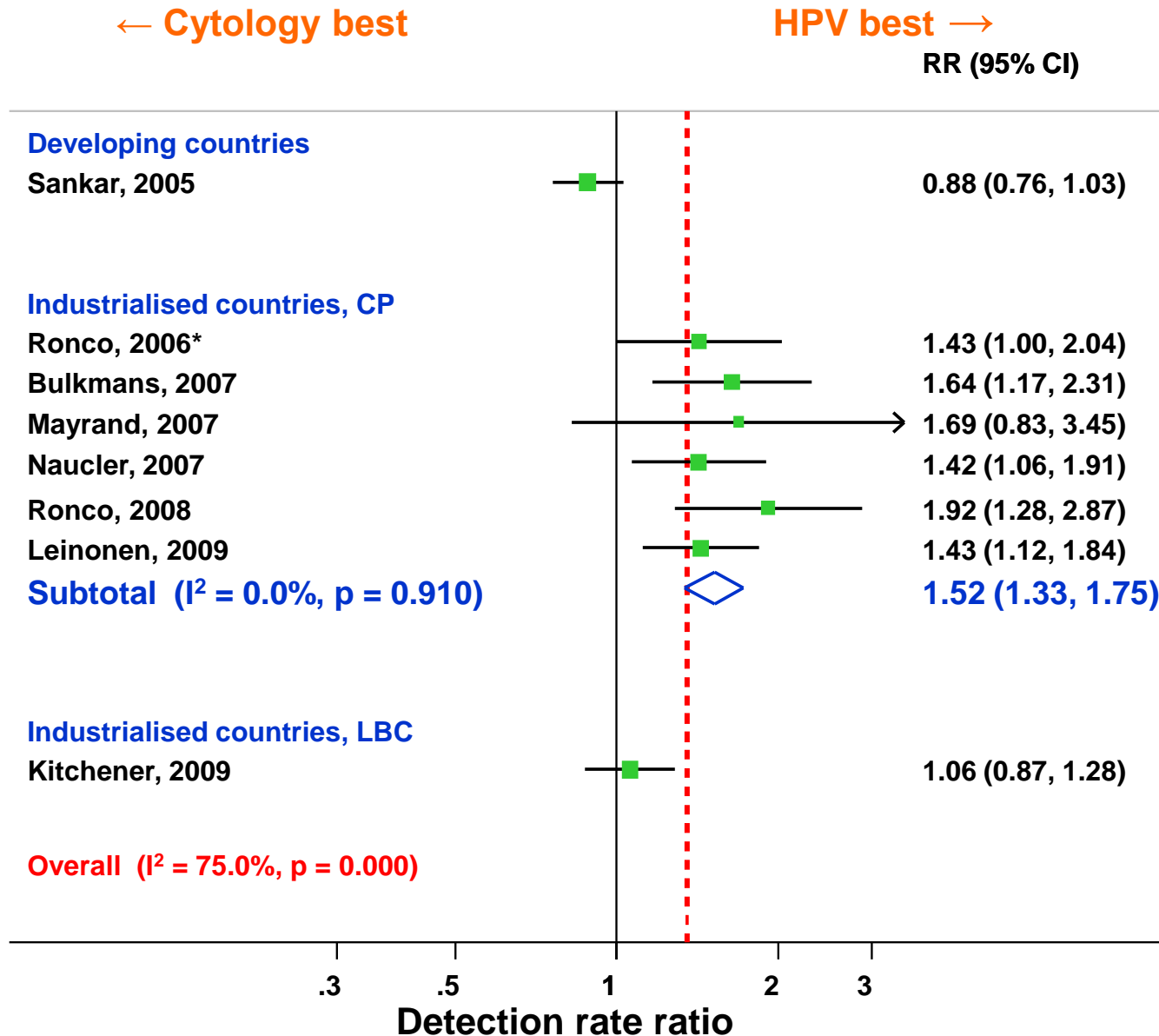
# Study characteristics

Trial	Age groups	Screening Tests
<b>India</b>	<b>30-59</b>	<b>VIA, HC2, CP vs no screening</b>
<b>Finland</b>	<b>30-60*</b>	<b>HPV-cyto-triage vs CP</b>
<b>Italy</b>	<b>20-34,<u>35-60</u></b>	<b>1) HC2 + LBC vs CP 2) HC2 vs CP</b>
<b>NL</b>	<b>30-60</b>	<b>GP5+/6+ PCR &amp; CP vs CP</b>
<b>Sweden</b>	<b>32-38</b>	<b>GP5+/6+ PCR &amp; CP vs CP</b>
<b>UK</b>	<b>20-64</b>	<b>HC2 + LBC vs LBC</b>
<b>Canada</b>	<b>30-69</b>	<b>HC2 + CP vs CP + HC2</b>

## Study characteristics (2)

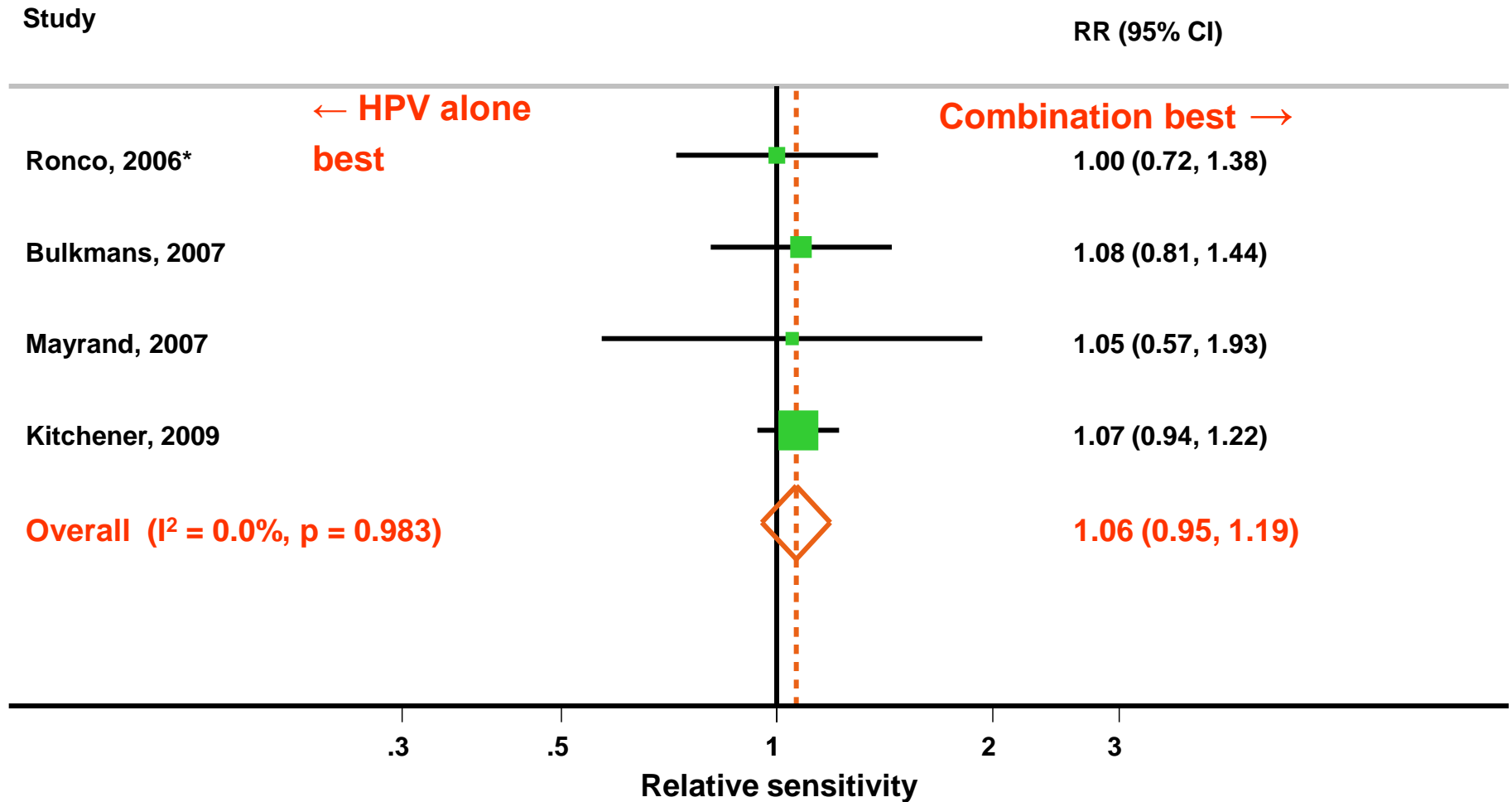
- **Various follow-up protocols for screen+**
- **Sometimes varying by age**
- **Different levels of blinding of gold standard verification**
- **For all trials: PPV, DR of CIN2, CIN3/AIS+**
- **4 trials: CIN3+ at 2<sup>nd</sup> screening round**
- **1 trial: I & M from cervical cancer**

# Baseline results: relative sensitivity for CIN2+



# (HPV & cyto) vs HPV alone

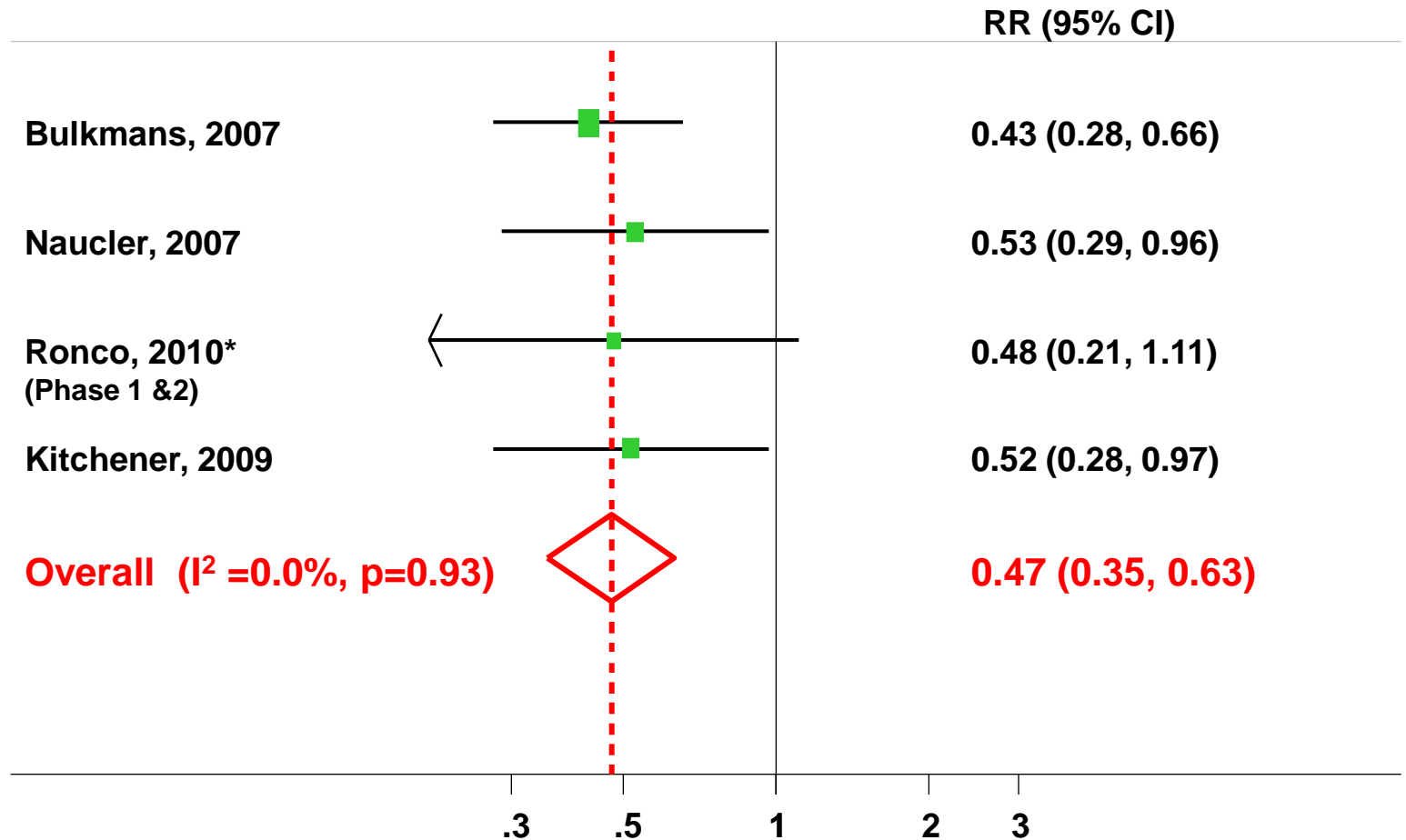
Detection of CIN2+, 1st screening round



Arbyn, Lancet Oncol 2009

# HPV- vs cytology based screening

## CIN3+ in 2<sup>nd</sup> round among in women with negative screen test at baseline



# Longitudinal results RCTs: HPV vs cyto screening

## Incidence of cervical cancer

- **Italian trial: HPV vs. Cyto group: 0 vs 9 cases**
- **Swedish RCT: 1 vs 5 cases**
- **Dutch trial: sign reduction in HPV arm**
- **Indian trial: -53% incidence (II+), -47% mortality**

Sankar 2009 NEJM; Ronco, Lancet Oncol 2010; Arbyn Lancet Oncol 2009; Naucner NEJM 2007;

## Conclusion: hrHPV DNA testing

- **More sensitive but less specific than cyto to detect CIN2+ & CIN3+**
- **Accuracy estimates less variable than cytology**
- **Combined screening (cyto + HPV) does not increase sensitivity but decreases specificity and generates costs & adverse effects**
- **Less CIN3+, less invasive cancer among women with negative HPV test at base line**

## Conclusion: hrHPV DNA testing

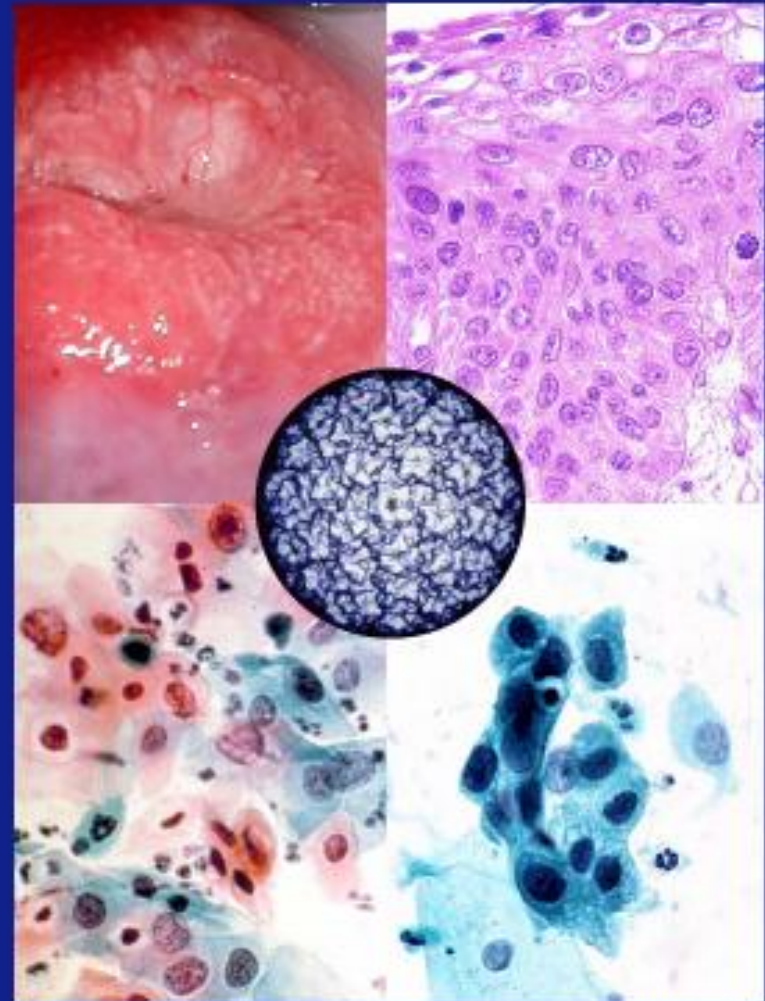
- **HPV screening with validated tests: good reproducibility, less ~ skilled human resources**
- **HPV screening will allow extending intervals, but this will require a high level of organisation otherwise explosion of follow-up examinations**
- **Reduced specificity requires appropriate triage (cytology, typing 16/18, p16, RNA-5 types, persistent (type-specific) infection**

# Conclusion: hrHPV DNA screening

- **Start HPV screening: not before 30 (35)Y**
- **Stop: 60/65Y**
- **Interval: 5-7Y**
- **Whatever the choice of future 1ary screening : the main factor of success will be the availability of a good organised and monitored system**

# Supplements to EU guidelines on HPV screening and vaccination

Ready by 2011



**European guidelines for quality assurance  
in cervical cancer screening** *Second Edition*



European Commission



## HPV Screening for Cervical Cancer in Rural India

Rengaswamy Sankaranarayanan, M.D., Bhagwan M. Nene, M.D., F.R.C.P., Surendra S. Shastri, M.D., Kasturi Jayant, M.Sc., Richard Muwonge, Ph.D., Atul M. Budukh, Ph.D., Sanjay Hingmire, B.Sc., Sylla G. Malvi, M.Sc., Ph.D., Ranjit Thorat, B.Sc., Ashok Kothari, M.D., Roshan Chinoy, M.D., Rohini Kelkar, M.D., Shubhada Kane, M.D., Sangeetha Desai, M.D., Vijay R. Keskar, M.S., Raghevendra Rajeshwarkar, M.D., Nandkumar Panse, B.Com., and Ketayun A. Dinshaw, M.D., F.R.C.R.

**Table 4. Incidence of Cervical Cancer and Rates of Death.\***

Variable	HPV Testing	Cytologic Testing	VIA	Control
Incidence of all cervical cancer — no.	127	152	157	118
Person-yr of follow-up — no.	268,185	250,523	267,326	247,895
Rate per 100,000 person-yr	47.4	60.7	58.7	47.6
Hazard ratio (95% CI)	1.05 (0.77–1.43)	1.34 (0.99–1.82)	1.30 (0.95–1.78)	1.00
Incidence of stage II or higher cervical cancer — no.	39	58	86	82
Person-yr of follow-up — no.	268,185	250,523	267,326	247,895
Rate per 100,000 person-yr	➡ 14.5	➡ 23.2	32.2	33.1
Hazard ratio (95% CI)	0.47 (0.32–0.69)	0.75 (0.51–1.10)	1.04 (0.72–1.49)	1.00
Death — no.	34	54	56	64
Person-yr of follow-up — no.	268,674	251,144	267,917	248,175
Rate per 100,000 person-yr	➡ 12.7	➡ 21.5	20.9	25.8
Hazard ratio (95% CI)	0.52 (0.33–0.83)	0.89 (0.62–1.27)	0.86 (0.60–1.25)	1.00

\* Rates and hazard ratios have been adjusted for age. Hazard ratios are for the comparison between each intervention group and the control group. CI denotes confidence interval, HPV human papillomavirus, and VIA visual inspection of the cervix with acetic acid.

The Lancet Oncology, [Volume 11, Issue 3](#), Pages 249 - 257, March 2010  
 doi:10.1016/S1470-2045(09)70360-2 [?](#) [Cite or Link Using DOI](#)  
 Published Online: 19 January 2010

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## Efficacy of human papillomavirus testing for the detection of invasive cervical cancers and cervical intraepithelial neoplasia: a randomised controlled trial

Dr [Guglielmo Ronco MD](#) [✉](#), [Paolo Giorgi-Rossi PhD](#) [f](#), [Francesca Carozzi PhD](#) [e](#), [Massimo Confortini PhD](#) [e](#), [Paolo Dalla Palma MD](#) [e](#), [Annarosa Del Mistro MD](#) [d](#), [Bruno Ghiringhello MD](#) [g](#), [Salvatore Girlando PhD](#) [e](#), [Anna Gillio-Tos PhD](#) [b](#), [Laura De Marco PhD](#) [b](#), [Carlo Naldoni MD](#) [i](#), [Paola Pierotti PhD](#) [h](#), [Raffaella Rizzolo MSc](#) [a](#), [Patrizia Schincaglia MD](#) [i](#), [Manuel Zorzi MSc](#) [d](#), [Marco Zappa MD](#) [e](#), [Nereo Segnan MD](#) [a](#), Prof [Jack Cuzick PhD](#) [k](#), the New Technologies for Cervical Cancer screening (NTCC) Working Group<sup>‡</sup>

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	<i>HPV group</i>	<i>Cytology group</i>	<i>p value</i>
All ages pooled			
Screening round 1	7	9	0.62
Screening round 2	0	9	0.004
Total over first 2 rounds	7	18	0.028

### Interpretation

HPV-based screening is more effective than cytology in preventing invasive cervical cancer, by detecting persistent high-grade lesions earlier and providing a longer low-risk period. However, in younger women, HPV screening leads to over-diagnosis of regressive CIN2.

# *SCREENING FOR HPV*

---

- ❖ Focused on increasing the overall proportion of women participating in cervical cancer screening procedures
  - ❖ HPV detection in self-obtained samples, such as vaginal and urine specimens
-

# HPV TEST

---

## 2. Which method;

### Target amplification

- PCR, type-specific PCR, real time PCR
- DNA microarrays
- DNA sequencing
- mRNA

### Signal amplification

- Hybrid capture
-

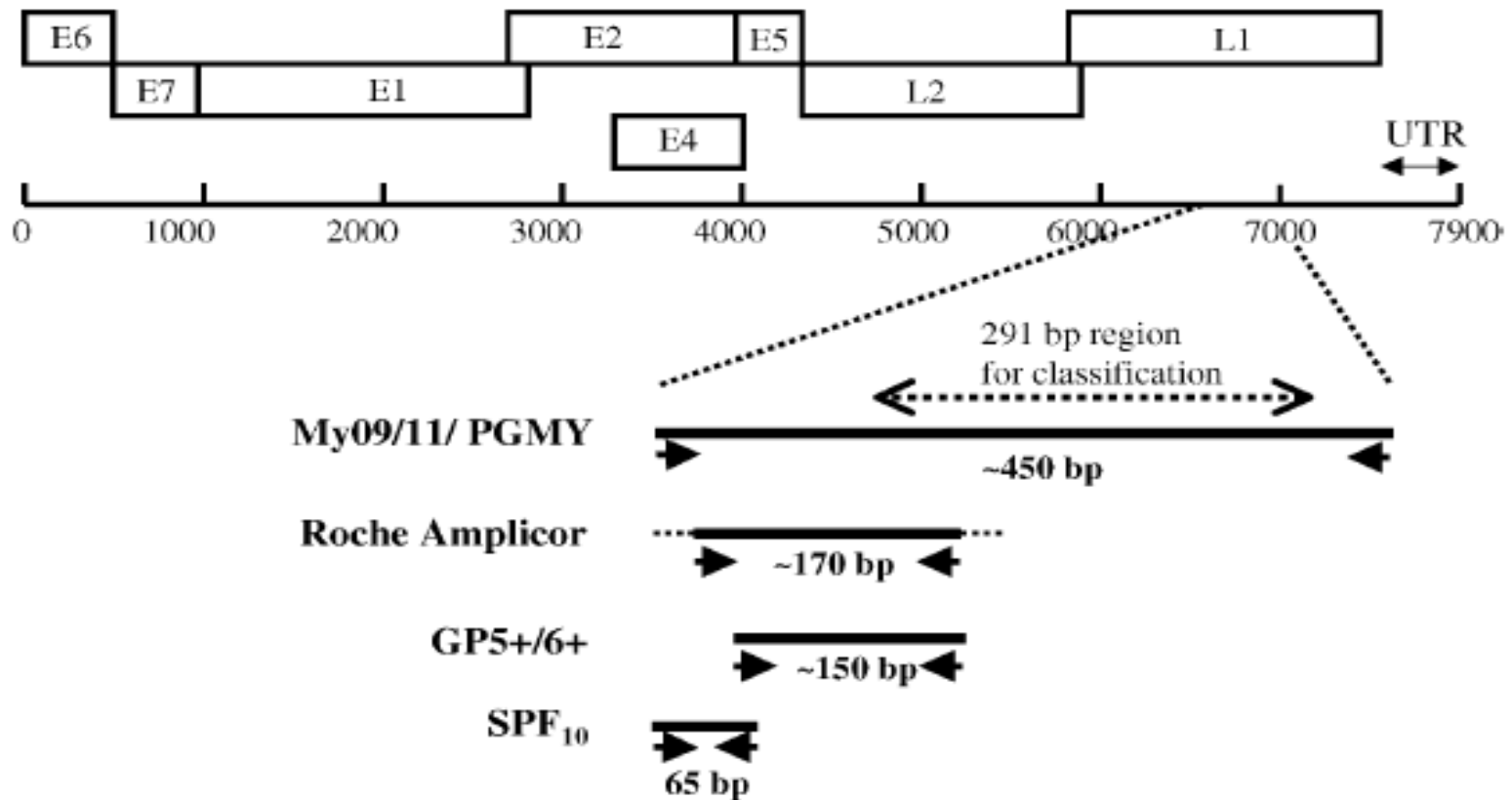
# Different HPV Tests commercially available

LR=χαμηλού κινδύνου, HR=υψηλού κινδύνου

Assay	Company	Detects	Can identify human papillomavirus 16 and 18
Abbott RealTime High Risk HPV	Abbott	14 genotypes (HR)	Yes
ProDect Chip HPV Typing	Bcs Biotech S.P.A.	24 genotypes (HR and LR)	Yes
NucliSENS EasyQ HPV	Biomerieux	5 genotypes (HR)- mRNA	Yes
HPV-DNA Qualitative Assay	Catch by Gene	15 genotypes (HR)	No
REBA HPV-ID	M&D, Inc.	25 genotypes (HR and LR)	Yes
LCD Array HPV 3.5	Chipron	32 genotypes (HR and LR)	Yes
Genpoint Tm HPV Test	Dako-Oxoid	13 genotypes (HR)	Yes
PapType	Genera Biosystems	14 genotypes (HR)	Yes
AID HPV Screening Kit	GenoID	49 genotypes (HR and LR)	No
AID HPV Typing Kit	GenoID	14 genotypes (HR)	Yes
AID STD Assay	GenoID	14 genotypes (HR)	No
Reveal HPV Real-Time HPV Detection Kit	GenoID	19 genotypes (HR and LR)	No
Array Papillomavirus	Genomica	35 genotypes (HR and LR)	Yes
Aptima	Gen-Probe	14 genotypes (HR)- mRNA	Yes
Papillocheck	Greiner BioOne	24 genotypes (HR and LR)	Yes
Cervista HPV HR Test <sup>^</sup>	Hologic	14 genotypes (HR)	Yes
Linear ArrayExtra HPV Genotyping Kit	Innogenetics	28 genotypes (HR and LR)	Yes
HPV OncoTect	Invirion Diagnostics	HR E6 and E7 mRNA expression in cells	No
Genesquare- HPV	Kurabo Industries	23 genotypes (HR and LR)	Yes
BIOPAP QTS HPV Kit	Loxo	6 genotypes (HR)	Yes
fHPV typing	molGENTIX	15 genotypes (HR and LR)	Yes
Luminex HPV Genotyping	Multimetrix/Progen	24 genotypes (HR and LR)	Yes
Care HPV	Qiagen	14 genotypes (HR)	No
Consensus HPV Typing Kit	Qiagen	18 genotypes (HR and LR)	Yes
Hybrid Capture 2 <sup>^</sup>	Qiagen	13 genotypes (HR)	No
Luminex HPV Assay	Qiagen	17 genotypes (HR and LR)	Yes
Amplicor HPV Test	Roche	13 genotypes (HR)	No
COBAS 4800 HPV Test	Roche	14 genotypes (HR)	Yes
Linear Array HPV Genotyping Test	Roche	37 genotypes (HR and LR)	Yes
Seeplex HPV Genotyping	Seegene	20 genotypes (HR and LR)	Yes
PCR Human Papillomavirus Detection Set	Takara Mirus Bio	7 genotypes (HR and LR)	Yes
Viroactiv	Virofem	8 genotypes (HR)	No

<sup>^</sup>Food and Drug Administration approved.

# PCR με χρήση εκκινητών συναίνεσης



*Int. J. Cancer*: **124**, 516–520 (2009)

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## FAST TRACK

# Guidelines for human papillomavirus DNA test requirements for primary cervical cancer screening in women 30 years and older

Chris J.L.M. Meijer<sup>1\*</sup>, Johannes Berkhof<sup>2</sup>, Philip E. Castle<sup>3</sup>, Albertus T. Hesselink<sup>1</sup>, Eduardo L. Franco<sup>4</sup>, Guglielmo Ronco<sup>5</sup>, Marc Arbyn<sup>6,7</sup>, F. Xavier Bosch<sup>8</sup>, Jack Cuzick<sup>9</sup>, Joakim Dillner<sup>10</sup>, Daniëlle A.M. Heideman<sup>1</sup> and Peter J.F. Snijders<sup>1</sup>

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<sup>3</sup>*Department of Cancer Epidemiology and Genetics, National Institute of Health, Washington, DC*

<sup>4</sup>*Division of Cancer Epidemiology, McGill University, Montreal, Canada*

<sup>5</sup>*Unit of Cancer Epidemiology, Centro per la prevenzione Oncologica, Turin, Italy*

<sup>6</sup>*Unit of Cancer Epidemiology, Scientific Institute of Public Health, Brussels, Belgium*

<sup>7</sup>*ECCG (European Cooperation on Development and Implementation of Cancer Screening and Prevention Guidelines), IARC, Lyon, France*

<sup>8</sup>*Servei d'epidemiologia, Institut Catala d'Oncologia (ICO), Hospitalet del llobregat, Barcelona, Spain*

<sup>9</sup>*Queen Mary's School of Medicine and Dentistry and Cancer Research UK, London, United Kingdom*

<sup>10</sup>*Department of Medical Microbiology University Hospital, Lund University, Malmö, Sweden*

# Screening – Post Vaccination

---

## # Longer screening intervals

- Requires knowledge of vaccination history
- Older women beyond vaccination age still need screening for 40+ years

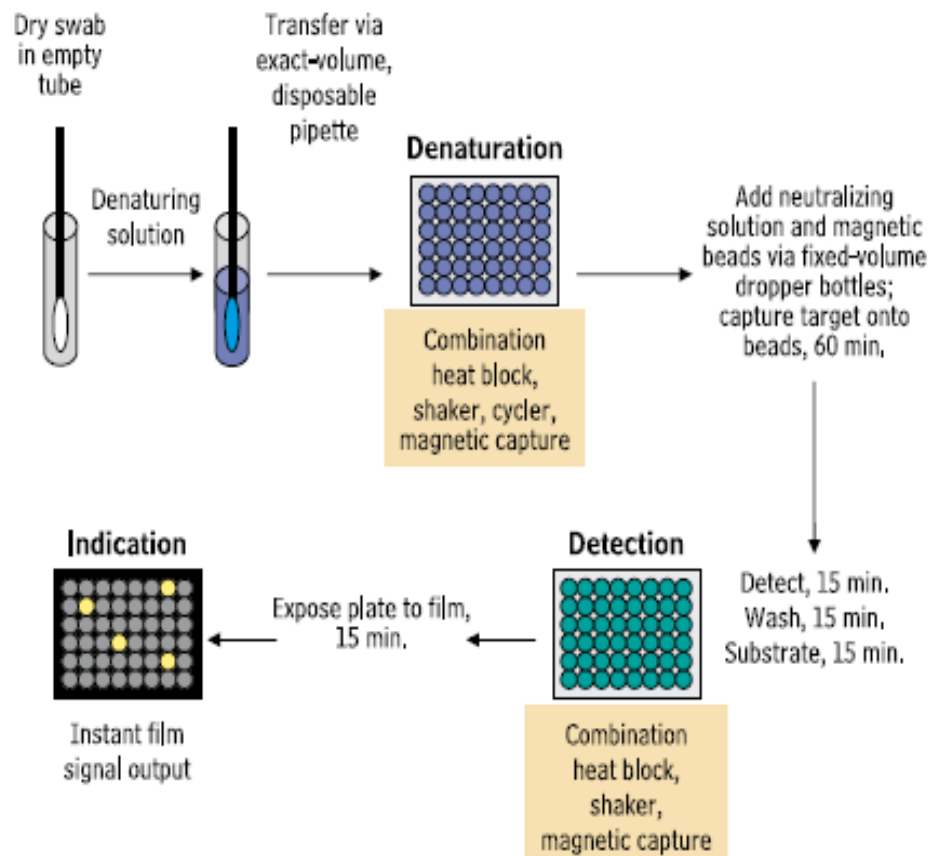
## # Self –Sampling ??

- Only sensitive with HPV testing
-

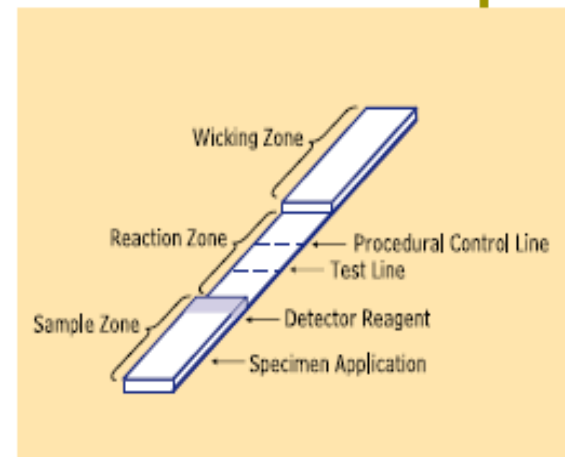
# *SELF-SAMPLE*

---

- ❖ Self-sampling is a less costly and non-invasive collection procedure
  - ❖ Numerous studies have reported lately that self-obtained samples of the anogenital tract in women were accurate and suitable for DNA testing
-



**Figure 1**  
Diagram showing the process used in the new Hybrid Capture® assay under development by Digene Corporation.



**Figure 2**  
Diagram of the basic components of the lateral flow test strip under development by PATH and ArborVita Corporation. An aliquot of the sample extract is placed on the sample zone from where it is then transported across the reaction zone. A readable test line indicates a positive result; the procedural control line verifies proper test function.


# HPV cervix test

## Test for precancerous changes to the cervix - £115.00

If you're unable or unwilling to have a traditional smear test then our postal test is a convenient and alternative way to get the information you need without having to go to a clinic.

Our postal HPV test uses a special tampon and can identify pre-cancerous changes to cells in your cervix caused by HPV subtypes 16,18,31,33 and 45. These subtypes cause over 80% of cervix cancer.

Discuss your HPV test results for free with our experts.

[Click to order](#) 


Information provided

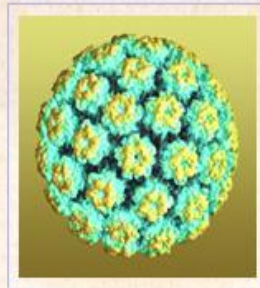
**“ DrThom says...**

a simple and convenient way to help protect yourself against cervical cancer

Home > disease management, early detection, screening > Screening HPV at Home

## Screening HPV at Home

March 16th, 2010  [Brian Mossop](#)



In Chapter 6 of The Decision Tree, "Screening for Everything", Thomas talks about the human papilloma virus (HPV), the virus that causes cervical cancer. Traditionally, doctors detected HPV by looking for irregular cells in the pap smear. But now, a cheap (\$5) test can determine if it is determining if it is determine the likelihood of cervical cancer.

One problem remains to be tested. However, a recent Journal shows that actually boost compliance rates. Roughly 28% of women consisted of a simple cervicovaginal lavage, effective 17% of women required to go into the doctor's office.

The HPV DNA test is primarily looking for the high-risk virus. A recent study claim that home screening kits have the same effectiveness as specifically looking for the aggressive virus.

Special thanks to Lindsay Crouse for bringing this to my attention. She brilliantly summed up the significance of home HPV testing.

## HPV Human Papillomavirus Home Test

Do you have the Human Papillomavirus? What can I take, What do I do?

Home About Sitemap Privacy Policy What You Should Know About HPV How Safe Is The HPV Vaccine? HPV Vaccine Side Effects

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**Belatrix Testing  
Services**

**Ask a Dr: Ovarian Cyst**

14 Doctors Are Online. Current Wait  
Time: 5 Minutes.

**HPV shot video**

Published by HPV Blogger October 18th, 2007 in HPV News. 0 Comments

A great video on the Human Papillomavirus.



**Gardasil - HPV vaccine**

Published by HPV Blogger October 18th, 2007 in HPV News. 0 Comments

GARDASIL is the only vaccine that may help guard against diseases that are

**EpiVax**

Immunogenicity Screening In silico, In  
vitro, In vivo assays

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Type and Wait to Search



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Αγορές **Δείτε Τώρα**

Latest

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Gardasil - HPV vaccine  
HPV Vaccine: What do you think?  
Cheap method to detect Cervical Cancer  
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Texas lawmakers reject HPV vaccine order  
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Gardasil  
Stanford HPV Awareness Campaign Video

HPV Articles

How Safe Is The HPV Vaccine  
HPV Vaccine Side Effects

## *HPV SCREENING DURING ANTENATAL VISITS*

---

- ❖ **increase participation**
  - ❖ **early detection**
  - ❖ **cost-effective for patients and the health services.**
-

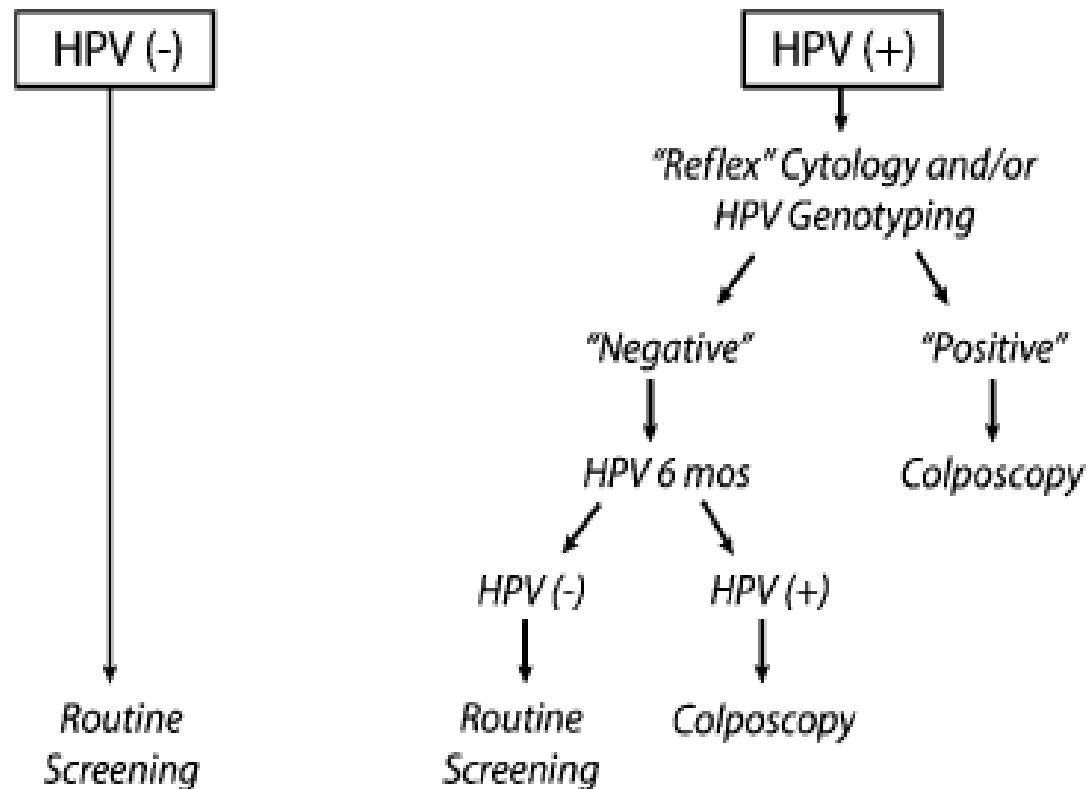
# HPV SCREENING

---

## Additional tests:

- # **HPV genotyping**  
*(16,18,... types)*
  - # **hrHPV mRNA analysis**
  - # **p16, L1, etc.** *(progression markers)*
  - # **Viral load analysis (?)**
-

## HPV Testing With “Reflex” Cytology and/or Genotyping



**FIGURE 4.** Novel HPV-based screening strategies incorporating either “reflex” cytology HPV genotyping, or a combination of both.

# *STUDY*

---

- ❖ screening for hrHPV types in first void urine and vaginal samples in 500 pregnant women attending the antenatal clinic
  - ❖ compare them with 250 non pregnant women hr HPV incidence rates from the same area in central Greece (University hospital of Larissa).
-

# *URINE SAMPLES*

---

- ❖ first void urine is the best sample because of the presence of more epithelial cells
  - ❖ Urinalysis and urine microscopy was performed
  - ❖ QIAamp Viral RNA Mini Kit (Qiagen Inc., Valencia, CA) was used for DNA extraction, which is considered to be more suitable for urine samples
-

# *METHODS*

---

- ❖ QIAamp DNA Mini Kit (Qiagen Inc., Valencia, CA) was used for DNA extraction
  - ❖ Vaginal swabs and urine samples were tested by an in house PCR method for the detection of hrHPV types 16,18,31 and 45
  - ❖ The quality of sample was validated by detection of the housekeeping gene beta-globin with an in-house PCR
-

# THE EIGHT MOST COMMON TYPES BY DECADES

**HPV types 16, 18, are the 1st and 2nd most common types over all time periods**

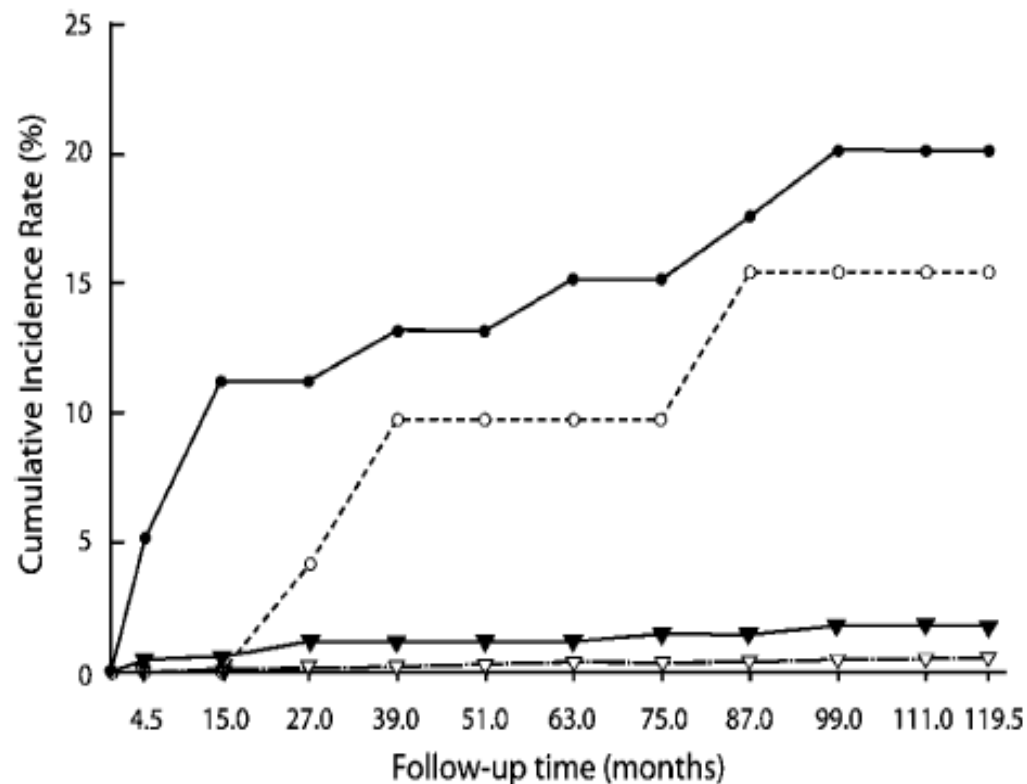
	1940-59	1960-69	1970-79	1980-89	1990-99	2000-9	GLOBAL
①	HPV 16	HPV 16	HPV 16	HPV 16	HPV 16	HPV 16	HPV 16
②	HPV 18	HPV 18	HPV 18	HPV 18	HPV 18	HPV 18	HPV 18
③	HPV 45	HPV 31	HPV 45	HPV 45	HPV 45	HPV 45	HPV 45
④	HPV 31,52	HPV 45	HPV 31,33	HPV 31	HPV 33	HPV 33	HPV 33
⑤	HPV 33	HPV 33	HPV 35	HPV 33	HPV 31	HPV 31	HPV 31
⑥	HPV 35	HPV 51	HPV 52	HPV 58	HPV 52	HPV 52	HPV 52
⑦	HPV 58	HPV 52	HPV 39	HPV 52	HPV 58	HPV 58	HPV 58
⑧	HPV 39	HPV 58	HPV 51	HPV 59	HPV 35	HPV 35	HPV 35

*Ranking using crude estimates*

*Alemán et al. In preparation*

# RESULTS

PREGNANT (500)	N of vaginal swabs	N of urine samples	% of total samples	NON PREGNANT (250)	N of vaginal swabs	N of urine samples	% of total samples
HPV 16	7	7	<b>1.4</b>	HPV 16	4	3	<b>1.6</b>
HPV 31	3	2	<b>0.6</b>	HPV 31	2	1	<b>0.8</b>
HPV 18	1	1	<b>0.2</b>	HPV 18	1	1	<b>0.4</b>
HPV 45	1	1	<b>0.2</b>	HPV 45	1	1	<b>0.4</b>



**FIGURE 3.** Impact of HPV status on the development of CIN 3 in women 30 years and older enrolled from Kaiser, Portland, OR. Solid circles represent women who were HPV 16 positive entry, open circles are those who were HPV 18 positive, solid triangles are women with other high-risk types of HPV, and open triangles are women who were high-risk HPV DNA negative. Modified from *J Natl Cancer Inst.* 2005; 97:1072–1079.

Epidemiology

## Establishment of the 1st World Health Organization international standards for human papillomavirus type 16 DNA and type 18 DNA

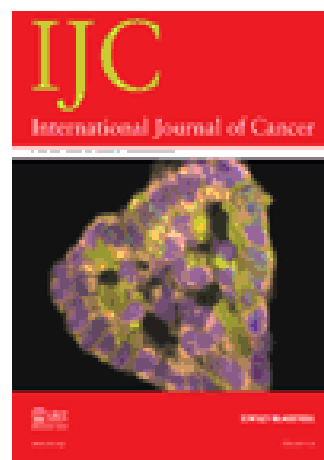
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# HPV Typing

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- # 14 High-risk types accepted
  - # HPV 16 most persistent and related to CIN3+
  - # HPV 18/45 also more related to CIN3+ and lesions in the endocervical canal not easily visualised on colposcopy
  - # Management of HPV 16 and possibly 18/45 should be more aggressive than for other HR types
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# *RESULTS*

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- ❖ HrHPV detection rate was similar in pregnant and non pregnant women
  - ❖ The urine/vaginal detection sensitivity was higher in pregnancy because first void urine samples from pregnant women contain two or more epithelial cells per field in urine microscopy
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# CONCLUSIONS

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- ❖ As expected, HPV16 was the most common type in our sample
  - ❖ Its prevalence is similar to that described for Southern Europe (1.2%)(De Sanjose et al., 2007) and for Greece (1.4%) (Agorastos et al., 2009)
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# *CONCLUSIONS*

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- ❖ Urine and vaginal samples are easily collected during antenatal visits
  - ❖ Useful for screening young women who are asymptomatic and for personal or cultural reasons would not participate in a cervical specimen screening program
  - ❖ Could be combined with Chlamydia and other STD screening
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