



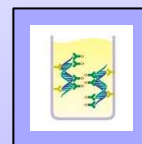
## *Workshop*

Programma Regionale di Screening per il Cervicocarcinoma  
(attività, risultati, prospettive)

*10 maggio 2010*

---

### *Qualità in laboratorio con HPV*



**Anna Gillio Tos**  
SSCVD Centro Unificato per lo Screening Cervico Vaginale  
Ospedale S. Giovanni Antica Sede

---

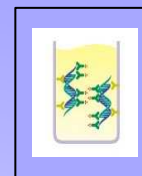
*Anna Gillio Tos: Workshop "Prog Reg Screening CC", Torino 10 mag 2010*

## **QUALITA' CON HPV-TESTING : 3 LIVELLI DI CONTROLLO**

**1. Accettazione campione di prelievo**



**2. Selezione del test analitico**



**3. Analisi Molecolare**  
**Riproducibilità e affidabilità del risultato :**  
**allestimento di controlli di qualità**  
**intra- ed inter- laboratorio**



## 1. Accettazione campione di prelievo



IL CAMPIONE RISULTA **INADEGUATO** IN CASO DI:

1. Assenza indicazioni corrette su etichetta
2. Assenza liquido di trasporto (Specimen Transport Medium)
3. Assenza spazzolino
4. Tappo mal chiuso
5. Asta spezzata troppo in basso
6. Asta spezzata troppo in alto
7. Presenza di 2 spazzolini
8. Presenza seconda porzione dell'asta spezzata

## 1. Accettazione campione di prelievo



### ETICHETTE BARCODE

#### SOFTWARE:

- Generazione dei codici a barre
- Lettura e abbinamento con i dati della donna

#### ETICHETTE:

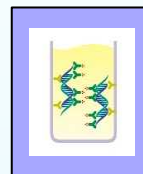
Ditta fornitrice: A.C.S.E. srl VIGONZA (PD)

- Etichette sintetiche di colore bianco per stampa a trasferimento termico fustellate in rotolo : confezioni da 10.500 etichette, cod. ET50-30MB
- verificata resistenza di colla e inchiostro alle colorazioni e all'incubazione in bagno maria a 65°C)
- tempi di consegna delle etichette : 15-20 giorni.

#### STAMPANTE:

- Stampante a trasferimento termico completa di alimentatore, ecc: cod. NP-210-TT
- Nastro carbone nero est., cod. E110-H68-74°

## 2. Selezione del test analitico



### Sensibilità relativa nelle tecniche di analisi degli acidi nucleici



Modificata da  
Hubbard R. A. '03

## ***CRITERI DI SELEZIONE DEL TEST DIAGNOSTICO***

- **Riproducibilità** →

- **Sensibilità**  
(NO falsi neg)

- **Specificità**  
(NO falsi pos)

HPV test per Screening Carcinoma Cervicale:

**- Hybrid Capture 2** (FDA, CE-IVD,  
in commercio)

**- PCR con primers GP5+ /6+**

2. C

## Performance test HYBRID CAPTURE 2 in screening cervicale (*NTCC trial*)

- **Riproducibilità** → **alta riproducibilità**
- **Sensibilità**  
(NO falsi neg)
  - 1. **analitica:** 1 pg/ml = 5000 copie hr- HPV
  - 2. **clinica:** > 95 % per CIN2+ (> 40% citologia)
- **Specificità**  
(NO falsi pos)
  - 1. **analitica:** > 97% per hr- HPV
  - 2. **clinica:** ~ 90 % per CIN2+ (96,3% citologia)

## ***CRITERI DI SELEZIONE DEL TEST HPV per SCREENING CERVICALE***

*Int. J. Cancer: 124, 516–520 (2009)*

© 2008 Wiley-Liss, Inc.

### **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>

**We caution against misguided attempts to increase the clinical sensitivity for HPV assays, as the adverse effect of a small gain in sensitivity will be a dramatic increase in the number of false positives (i.e. hrHPV positives without  $\geq$ CIN2). Given the low prevalence of  $\geq$ CIN2 in the screened populations, even small reductions in clinical specificity will have dramatic effects on the number of unneeded follow up procedures and associated costs.**



## ***CRITERI DI SELEZIONE DEL TEST HPV per SCREENING CERVICALE***

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

Chris J.L.M. Meijer et al., *Int J Cancer* : 124, 516-520 (2009)

The candidate test should have a clinical sensitivity for  $\geq$ CIN2 not less than 90% of the clinical sensitivity of the hc2 in women of at least 30 years. This recommendation is based on recent data meta-analyses that reported a pooled sensitivity for hc2 of 97.9% (95%CI: 95.9%-99.9%) in primary screenig in Europe and North America<sup>6</sup> and a pooled sensitivity of hc2 and GP5+/6+ PCR in European studies of 96.1% (95%CI: 94.2%-97.4%).<sup>11</sup>

... a clinical specificity for  $\geq$ CIN2 of the candidate test not less than 98% of the clinical specificity of the hc2 in women of at least 30 years of age. ... In Europe the pooled specificity was 91.3% ...

### 3. **Analisi Molecolare: Riproducibilità e affidabilità allestimento di controlli di qualità INTRA- ed INTER- laboratorio**



#### Adeguatezza del Laboratorio

1. **Struttura:** aree separate per gestione/preparazione dei campioni biologici, preparazione reagenti e esecuzione dei test di rilevazione di HPV-DNA → **NO CONTAMINAZIONE**
2. **Procedure:** in accordo con SOP (Standard Operation Procedures)  
e con GLP (Good Laboratories Practice)
3. **Monitoraggio della Qualità del test:** allestimento programmi di controllo di qualità intra- ed inter-laboratorio  
*Carozzi et al. Am J Clin Pathol 2005;124:716  
(Trial NTCC:)*

### 3. Analisi Molecolare: Riproducibilità e affidabilità monitoraggio con controlli di qualità INTRA- laboratorio (CQI)



Riproducibilità test HC2 in sedute diverse e con operatori diversi

- Calibratori negativi (3 replic): **CN**
- Calibratori positivi (3 replic.): **CP**
- Controllo Low Risk HPV: **Q-LR**
- Controllo High Risk HPV: **Q-HR**
- 1 aliquota **CP** sedute precedenti
- 1 campione seduta precedente  
(Ratio RLU/CO: 2-10)

#### VALORI ATTESI

RLU	Ratio RLU/CP	CV replic.
10 -250	<1.0	< 25%
CP/CN: 2-15	1.0	< 25%
<150	<1.0	
>300	2 – 8	
	1.0	
	2-10	

### 3. Analisi Molecolare: Riproducibilità e affidabilità monitoraggio con controlli di qualità INTRA- laboratorio (CQI)



Es: inadeguatezza H<sub>2</sub>O deionizzata

	ATTESO	H2O DISTIL	H2O DEION	ATTESO	H2O DISTIL	H2O DEION
	RLU	RLU	RLU	Ratio RLU/CP	Ratio RLU/CP	Ratio RLU/CP
• <b>CN</b>	10 -250	54	122	<1.0	0.11	0.23
• <b>CP</b>	CP/CN: 2-15	455	522	1.0	1.0	1.0
• <b>Q-LR</b>	<150	65	542	<1.0	0.14	1.05
• <b>Q-HR</b>	>300	2422	3320	2 - 8	5.3	6.3
• <b>1 camp ripetuto</b>		63	560		0.13	1.1

### **3. Analisi Molecolare: Riproducibilità e affidabilità monitoraggio con controlli di qualità INTER- laboratorio**



#### **1. Confronto periodico inter-laboratorio**

- **Valori Medi calibratori e controlli interni**
- **Percentuali positività ad HPV**

#### **2. Partecipazione a programmi VEQ (Valutazione Esterna Qualità)**

**Un ente esterno prepara e spedisce a differenti laboratori lo stesso panel di campioni, restituendo risultati di performance specifiche e cumulative secondo limiti di accettabilità del risultato predefiniti ed elaborando una graduatoria di merito dei partecipanti.**

### 3. Analisi Molecolare: Riproducibilità e affidabilità monitoraggio con controlli di qualità INTER- laboratorio (VEQ)



QCMD 2009 Human Papillomavirus DNA EQA Pilot Study (Nanogen)  
Thank you for participating in this QCMD EQA Programme.

#### Report sens.analitica con HC2

Sample	Sample Content *	Sample conc. **	Sample Status
HPVDNA09-06	HPV 16 in CaSki	160,000	Frequently detected
HPVDNA09-08	HPV 16 in CaSki	80,000	Frequently detected
HPVDNA09-02	HPV 16 in CaSki	40,000	Detected
HPVDNA09-01	HPV 16 in CaSki	20,000	Detected
HPVDNA09-09	HPV 16 in CaSki	10,000	Negative
HPVDNA09-04	HPV 16 in CaSki	5,000	Negative
HPVDNA09-10	HPV 16 in CaSki	1,250	Negative
HPVDNA09-03	HPV 18 in HeLa	400,000	Detected
HPVDNA09-07	HPV 67 in Cc11	40,000	Detected
HPVDNA09-05	HPV Negative		Negative

\* PreservCyt A standard methanol-based, buffered preservative solution.

\*\* Copies/4ml.

Some commercial kits do not detect HPV type 67 and thus would consider this sample as HPV-negative.

#### Report sens.clinica

Sample Status
Frequently detected
Frequently detected
Detected
Detected
Negative
Negative
Negative
Detected
Detected
Negative

#### Report sens.analitica con PCR

Sample Status
Frequently detected
Frequently detected
Detected
Detected
Detected
Infrequently detected
Infrequently detected
Detected
Detected
Negative

### 3. Analisi Molecolare: Riproducibilità e affidabilità monitoraggio con controlli di qualità INTER- laboratorio (VEQ)

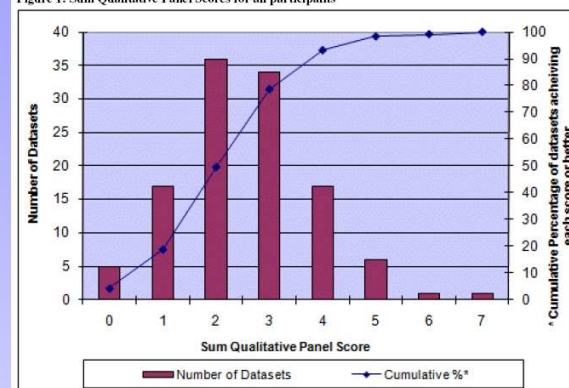


#### VEQ: analisi dei risultati

Table 2: Your laboratory's qualitative results and performance scores

Sample	Sample Content	Qualitative		
		Sample Status	Your qualitative result	Your qualitative score
HPVDNA09-06	HPV 16 in CaSki	Frequently detected	positive	0
HPVDNA09-08	HPV 16 in CaSki	Frequently detected	positive	0
HPVDNA09-02	HPV 16 in CaSki	Detected	positive	0
HPVDNA09-01	HPV 16 in CaSki	Detected	positive	0
HPVDNA09-09	HPV 16 in CaSki	Negative	negative	0
HPVDNA09-04	HPV 16 in CaSki	Negative	negative	0
HPVDNA09-10	HPV 16 in CaSki	Negative	negative	0
HPVDNA09-03	HPV 18 in HeLa	Detected	positive	0
HPVDNA09-07	HPV 67 in Cc11	Detected	negative	1
HPVDNA09-05	HPV Negative	Negative	negative	0
Sum Qualitative Panel Score				1

Figure 1: Sum Qualitative Panel Scores for all participants



The number of Qualitative datasets analysed : 117  
The sum of the Qualitative Panel Score for your dataset is : 1  
This score (or better) was achieved by : 18.8 % of all datasets

0 = corretto; 1 = errore

0 = corretto

> 1 = errore

### **3. Analisi Molecolare: Riproducibilità e affidabilità monitoraggio con controlli di qualità INTER- laboratorio (VEQ)**



#### **Gruppo di lavoro Gisci su CQ: progetto allestimento VEQ su HC2**

##### **Obiettivi:**

1. Accuratezza risultato pos e neg
2. Precisione del risultato
3. Inserimento di campioni reali, non solo di campioni sintetici
4. Costo VEQ accessibile/nullo



Grazie per l'attenzione



## References

- Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV, Snijders PJ, Meijer CJ. International Agency for Research on Cancer Multicenter Cervical Cancer Study Group. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med* 2003;348:518-27.
- IARC Monographs on the evaluation of carcinogenic risks to humans. Human Papillomaviruses. International Agency for Research on Cancer. Vol. 90. Geneva, Switzerland: WHO press, 2007.
- Smith JS, Lindsay L, Hoots B, Keys J, Franceschi S, Winer R, Clifford GM. Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. *Int J Cancer* 2007;121:621-32.
- Schiffman M, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S. Human papillomavirus and cervical cancer. *Lancet* 2007;370:890-907.
- Snijders PJ, Steenbergen RD, Heideman DA, Meijer CJ. HPV-mediated cervical carcinogenesis: concepts and clinical implications. *J Pathol* 2006;208:152-64.
- Arbyn M, Sasieni P, Meijer CJ, Clavel C, Koliopoulos G, Dillner J. Chapter 9: Clinical applications of HPV testing: A summary of meta-analyses. *Vaccine* 2006;24 (Suppl 3):S78-S89.
- Bulk S, Bulkman NW, Berkhof J, Rozendaal L, AJ PB, Verheijen RH, Snijders PJ, Meijer CJ. Risk of high-grade cervical intra-epithelial neoplasia based on cytology and high-risk HPV testing at baseline and at 6-months. *Int J Cancer* 2007;121:361-7.
- Romco G, Segnan N, Giorgi-Rossi P, Zappa M, Casadei GP, Carozzi F, Dalla Palma P, Del Mistro A, Folicardi S, Gillio-Tos A, Nardo G, Naldoni C, et al. New Technologies for Cervical Cancer Working Group. Human Papillomavirus testing and liquid-based cytology: results at recruitment from the New Technologies for Cervical Cancer randomized controlled trial. *J Natl Cancer Inst* 2006;98:765-74.
- Romco G, Giorgi-Rossi P, Carozzi F, Dalla Palma P, Del Mistro A, De Marco L, de Lillo M, Naldoni C, Pierotti P, Rizzolo R, Segnan N, Schincaglia, et al. New Technologies for Cervical Cancer Working Group. Human papillomavirus testing and liquid-based cytology in primary screening of women younger than 35 years: results at recruitment for a randomised controlled trial. *Lancet Oncol* 2006;7:547-55.
- Mayrand MH, Duarte-Franco E, Rodrigues I, Walter SD, Hanley J, Ferenczy A, Ratnam S, Coutlée F, Franco EL, Canadian Cervical cancer Screening Trial Study Group. Human papillomavirus DNA versus Papanicolaou screening tests for cervical cancer. *N Engl J Med* 2007;357:1579-88.
- Cuzick J, Clavel C, Petry KU, Meijer CJ, Hoyer H, Ratnam S, Szarewski A, Birembaut P, Kulasingam S, Sasieni P, Ifner T. Overview of the European and North American studies on HPV testing in primary cervical cancer screening. *Int J Cancer* 2006;119:1095-101.
- Schiffman M, Khan MJ, Solomon D, Herrero R, Wacholder S, Hildesheim A, Rodriguez AC, Bratti MC, Wheeler CM, Burk RD, PEG Group, ALTS Group. A study of the impact of adding HPV types to cervical cancer screening and triage tests. *J Natl Cancer Inst* 2005;97:147-50.
- Stoler MH, Castle PE, Solomon D, Schiffman M, American Society for Colposcopy and Cervical Pathology. The expanded use of HPV testing in gynecologic practice per ASCCP-guided management requires the use of well-validated assays. *Am J Clin Pathol* 2007;127:335-7.
- Hesselink AT, van Ham MAPC, Heideman DAM, Groothuisink ZMA, Rozendaal L, Berkhof J, van Kemenade FJ, Massuger LAFG, Melchers WJG, Meijer CJLM, Snijders PJF. Comparison of GP5+/6+-PCR and SPF10-line blot assays for detection of high-risk human papillomavirus in samples from women with normal cytology results who develop grade 3 cervical intraepithelial neoplasia. *J Clin Microbiol* 2008;46:3215-21.
- Hesselink AT, van den Brule AJC, Brink AATP, Berkhof J, van Kemenade FJ, Verheijen RH, Snijders PJF. Comparison of hybrid capture 2 with in situ hybridization for the detection of high-risk human papillomavirus in liquid-based cervical samples. *Cancer Cytopathology* 2004;102:11-18.
- Gravitt PE, Burk RD, Lorincz A, Herrero R, Hildesheim A, Sherman ME, Bratti MC, Rodriguez AC, Helzlsouer KJ, Schiffman M. A comparison between real-time polymerase chain reaction and hybrid capture 2 for human papillomavirus DNA quantitation. *Cancer Epidemiol Biomarkers Prev* 2003;12:477-84.
- Naucle P, Ryd W, Tornberg S, Strand A, Wadell G, Elfgrén K, Radberg T, Strander B, Forslund O, Hanson BG, Rylander E, Dillner J. Human papillomavirus and Papanicolaou tests to screen for cervical cancer. *N Engl J Med* 2007;357:1589-97.
- Bulkman N, Berkhof J, Rozendaal L, van Kemenade F, Boeke A, Bulk S, Voorhorst FJ, Verheijen RH, van Grooten K, Boon ME, Ruijnga W, van Ballegooijen M, et al. Human papillomavirus DNA testing for the detection of cervical intraepithelial neoplasia grade 3 and cancer: 5-year follow-up of a randomised controlled implementation trial. *Lancet* 2007;370:1764-72.

19. Ronco G, Giorgi-Rossi P, Carozzi F, Confortini M, Dalla Palma P, Del Mistro A, Gillio-Tos A, Mimucci D, Naldini C, Rizzolo R, Schincaglia P, Volante R, et al. New Technologies for Cervical Cancer Screening Working Group. Results at recruitment from a randomized controlled trial comparing human papillomavirus testing alone with conventional cytology as the primary cervical cancer screening test. *J Natl Cancer Inst* 2008;100:492-501.
20. Sherman ME, Lorincz AT, Scott DR, Wacholder S, Castle PE, Glass AG, Mielzynska-Lolmas I, Rush BB, Schiffman M. Baseline cytology, human papillomavirus testing, and risk for cervical neoplasia: a 10-year cohort analysis. *J Natl Cancer Inst* 2003;95:46-52.
21. Castle PE, Schiffman M, Burk RD, Wacholder S, Hildesheim A, Herrero R, Bratti MC, Sherman ME, Lorincz A. Restricted cross-reactivity of hybrid capture 2 with nononcogenic human papillomavirus types. *Cancer Epidemiol Biomarkers Prev* 2002;11:1394-9.
22. Hesselink AT, Bulkman NW, Berkhof J, Lorincz AT, Meijer CJ, Snijders PJ. Cross-sectional comparison of an automated hybrid capture 2 assay and the consensus GP5+/6+ PCR method in a population-based cervical screening program. *J Clin Microbiol* 2006;44:3680-5.
23. Coglian V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F. WHO International Agency for Research on Cancer Monograph Working Group. Carcinogenicity of human papillomaviruses. *Lancet Oncol* 2005;6:204.
24. Muñoz N, Bosch FX, Castellsagué X, Díaz M, de Sanjose S, Hammouda D, Shah KV, Meijer CJ. Against which human papillomavirus types shall we vaccinate and screen? The international perspective. *Int J Cancer* 2004;111:278-85.
25. Snijders PJF, Hogewoning CJA, Hesselink AT, Berkhof J, Voorhorst FJ, Bleeker MCG, Meijer CJLM. Determination of viral load thresholds in cervical scrapings to rule out CIN 3 in HPV 16, 18, 31 and 33-positive women with normal cytology. *Int J Cancer* 2006;119:1102-7.
26. Arbyn M, Anttila A, Jordan J, Ronco G, Schenck U, Segnan N, Wiener H, Daniel J, von Karsa L, editors. European Guidelines for Quality Assurance in Cervical Cancer Screening, 2nd edn. Luxembourg: Office for Official Publications of the European Communities, 2008. pp. 1-291.
27. Cuzick J, Szarewski A, Cubie H, Hulman G, Kitchener H, Luesley D, McGowan E, Menon U, Terry G, Edwards R, Brooks C, Desai M, et al. Management of women who test positive for high-risk types of human papillomavirus: the HART study. *Lancet* 2003;362:1871-6.
28. Castle PE, Wheeler CM, Solomon D, Schiffman M, Peyton CL, ALTS Group. Interlaboratory reliability of Hybrid Capture 2. *Am J Clin Pathol* 2004;122:238-45.
29. Carozzi FM, Del Mistro A, Confortini M, Sani C, Paliti D, Trevisan R, De Marco L, Tos AG, Girlando S, Palma PD, Pellegrini A, Schiombi ML, et al. Reproducibility of HPV DNA Testing by Hybrid Capture 2 in a Screening Setting. *Am J Clin Pathol* 2005;124:716-21.
30. Jacobs MV, Snijders PJ, Voorhorst FJ, Dillner J, Forslund O, Johansson B, von Knebel Doeberitz M, Meijer CJ, Meyer T, Nindl I, Pfister H, Stockfleth E, et al. Reliable high risk HPV DNA testing by polymerase chain reaction: an intermethod and intramethod comparison. *J Clin Pathol* 1999;52:498-503.
31. Tang NS, Tang ML, Chan IS. On tests of equivalence via non-unity relative risk for matched-pair design. *Stat Med* 2003;22:1217-33.
32. Quint WG, Pagliusi SR, Lelie N, De Villiers E-M, Wheeler CM. WHO Human Papillomavirus DNA International Collaborative Study Group. Results of the first world health organization international collaborative study of detection of human papillomavirus DNA. *J Clin Microbiol* 2006;44:571-9.
33. Dillner L, Dillner J. International quality assurance of human papillomavirus testing. *Cent Eur J Public Health* 2008;16:S18-S20.
34. Herbert A, Arbyn M, Bergeron C. Why CIN3 and CIN2 should be distinguished on histological reports. *Cytopathology* 2008;19:63-4.

## Appendix

### Non-inferiority test

Suppose  $n$  samples have been tested with the new test and the hc2 reference test. The results are presented in the following Table A1.

TABLE A1 - TEST RESULTS

	hc2 +	hc2 -	Total
New test +	a	b	a+b
New test -	c	d	c+d
Total	a+c	b+d	n

Under the null hypothesis, the relative sensitivity (when comparing the new test to hc2) is  $\delta_0$  and under the alternative hypothesis, the relative sensitivity is greater than  $\delta_0$ . According to the present guidelines  $\delta_0$  should be set to 0.90 for sensitivity and to 0.98 for specificity. The test statistic is defined as

$$T = \frac{a + b - (a + c)\delta_0}{\sqrt{n\{(1 + \delta_0)f + (a + b + c)(\delta_0 - 1)/n\}}},$$

where

$$f = \frac{\sqrt{B^2 - 4AC} - B}{2A},$$

with  $A = n(1 + \delta_0)$ ,  $B = (a + c)\delta_0^2 - (a + b + 2c)$  and  $C = c(1 - \delta_0)(a + b + c)/n$ . The null hypothesis is rejected at nominal significance level  $\alpha$  if  $T$  is equal to or greater than the  $100 \times (1 - \alpha)$  percentile point of the standard normal distribution ( $T$  is interpreted as a  $z$  statistic).