# VALidation of tests usable as a first step TRIage to manage hrHPV Positive women

## VALTRIHP: Nuovi Test

Clementina Cocuzza

*Università degli Studi di Milano – Bicocca*

**HPV DNA test -** esame di **riferimento per le donne di età compresa tra i 30 e i 65 anni,** da ripetersi **ogni 5 anni.**

Per le donne tra i **25 e i 29 anni l'esame di riferimento** rimane **il Pap-test,** da effettuarsi **ogni 3 anni**.


## SCREENING PRIMARIO HPV

- Dove è stato adottato, **lo screening primario con l'HPV test** ha offerto **miglioramenti** nella prevenzione del carcinoma della cervice uterina

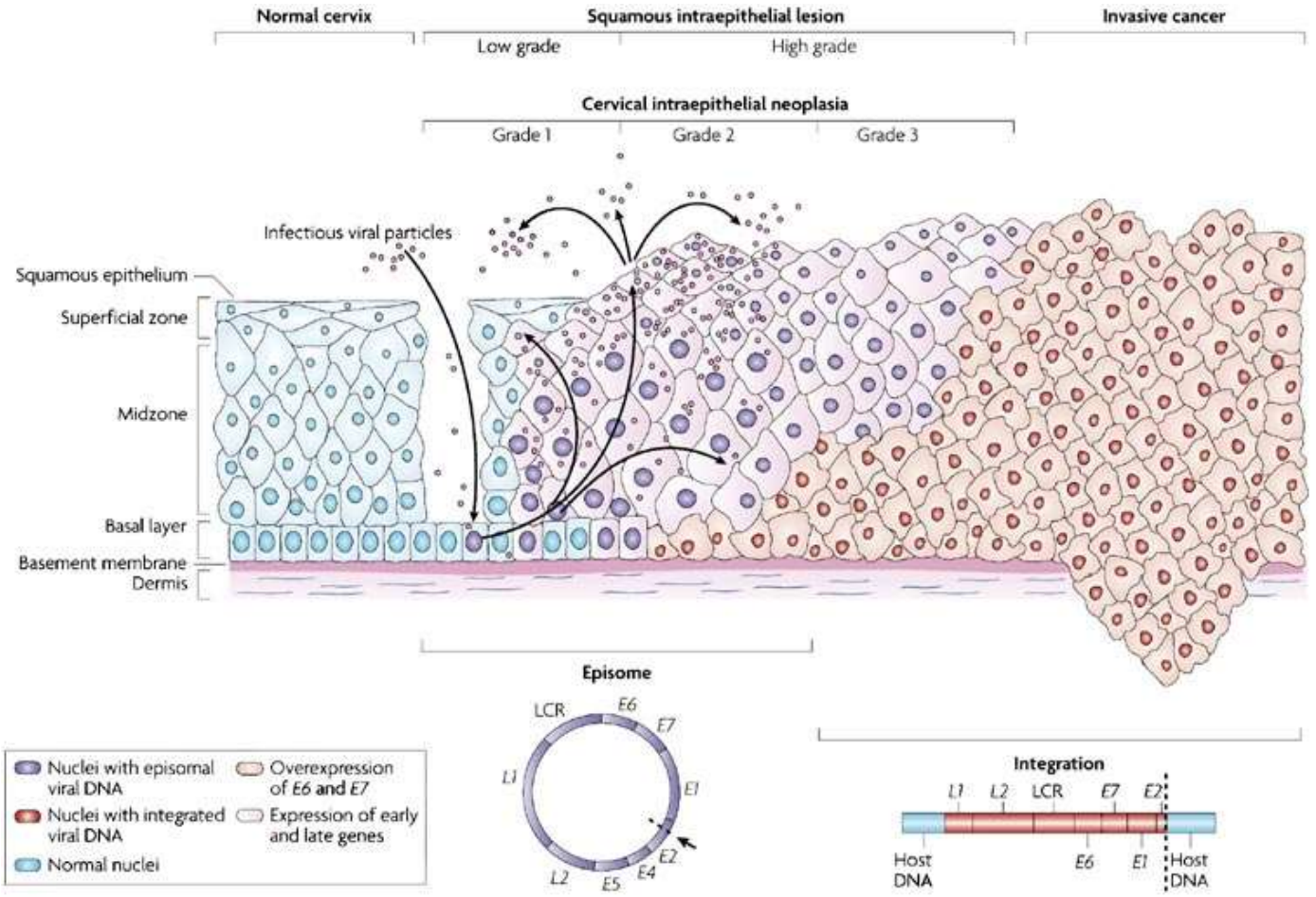
- Alcuni bisogni rimangono insoddisfatti:

  - il numero di **donne "non responder"** che per diversi motivi, sia personali che culturali, non aderiscono ai programmi di prevenzione;

  - **la scarsa specificità clinica degli attuali HPV-Test, non in grado di discriminare** le **infezioni transienti** da quelle **persistenti** ovvero le infezioni che determinano l'evoluzione in lesioni precancerose e cancerose.

**TRIAGE delle donne HPV-positive allo Screening Primario**

- **Indagini di Microscopia**
  - **Citologia**
  - **Dual staining P16/Ki-67**
- **Indagini Molecolari**
  - **Genotipizzazione - parziale vs completa**
  - **Quantificazione virale e trascritti oncogeni (mRNA E6/E7)**
  - **Marker di Metilazione – ospite e/o virali**




Nature Reviews | Cancer

nature REVIEWS CANCER

Woodman *et al*. *Nature Reviews Cancer* **7**, 11–22 (January 2007) | doi:10.1038/nrc2050

# Biomarker molecolari per il TRIAGE di donne HPV-positive

- **Genotipizzazione completa dei 12 genotipi di HPV ad alto rischio**

  ➢NTCC Study: Genotyping in 2.255 HPV+ve women (baseline and 3yrs FU)
  HPV types divided in 3 clusters based on PPV for CIN2+ and CIN3

| cluster | genotipo | PPV (%) CIN2+ | cluster | genotipo | PPV (%) CIN3+ |
|---|---|---|---|---|---|
| A | HPV33 | 22,39 | A | HPV33 | 11,94 |
| | HPV16 | 15,81 | | HPV16 | 8,78 |
| | HPV35 | 15,82 | | HPV35 | 8,87 |
| B | HPV31 | 13,99 | B | HPV58 | 8,10 |
| | HPV52 | 13,51 | | HPV59 | 7,80 |
| | **HPV18** | **12,68** | | HPV68 | 7,58 |
| | HPV59 | 12,46 | | HPV31 | 6,43 |
| | HPV58 | 12,00 | | **HPV18** | **5,93** |
| | | | | HPV52 | 5,74 |
| C | HPV39, HPV51, HPV56, HPV45, **HPV68** | 11,64-9,61 | C | HPV39, HPV45, HPV51, HPV56 | 5,51-4,57 |

Del Mistro et al, Int J Cancer 2018;143:333-342

Published OnlineFirst September 5, 2019; DOI: 10.1158/1055-9965.EPI-19-0239

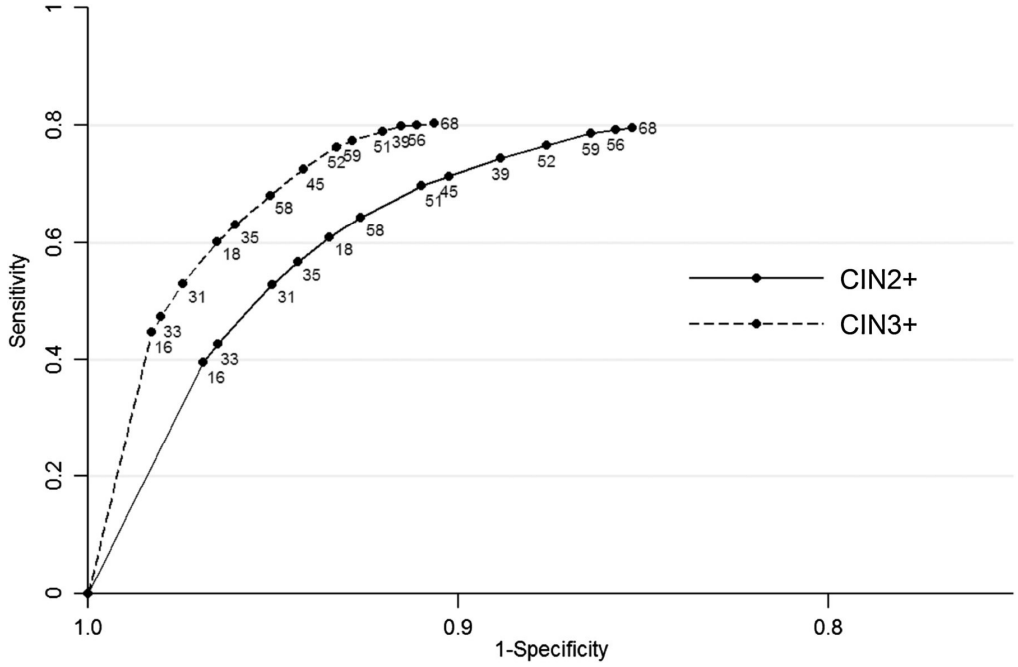
Research Article

Cancer Epidemiology, Biomarkers & Prevention

# Role of HPV Genotype, Multiple Infections, and Viral Load on the Risk of High-Grade Cervical Neoplasia

Rachael Adcock[1], Jack Cuzick[1], William C. Hunt[2], Ruth M. McDonald[2], and Cosette M. Wheeler[2]; for the New Mexico HPV Pap Registry Steering Committee

47,120 women undergoing cervical screening tested for 13 HR-HPV genotypes followed up for 3 years




## Hierarchical PPVs for 13 hrHPV genotypes stratified by viral load groups [high, intermediate, and low] for CIN2+ and CIN3+, weighted to the state wide population of women.

| | PPV (%) by viral load | | | |
|---|---|---|---|---|
| | CIN2+ | | | |
| HPV type | Low | Intermediate | High | *P* value for trend[a] |
| *Any hrHPV*[b] | 2.38 | 4.46 | 9.28 | 5.3E-169 |
| 16 | 3.90 | 6.61 | 17.62 | 1.2E-72 |
| 33 | 4.43 | 9.41 | 10.29 | 1.1E-03 |
| 31 | 4.08 | 8.93 | 10.76 | 1.2E-12 |
| 35 | 3.62 | 6.03 | 10.10 | 1.2E-05 |
| 18 | 2.07 | 2.83 | 7.70 | 2.5E-09 |
| 58 | 1.18 | 2.22 | 6.20 | 4.0E-09 |
| 51 | 2.31 | 4.40 | 4.50 | 1.2E-04 |
| 45 | 1.32 | 2.35 | 3.38 | 0.01 |
| 39 | 1.99 | 2.78 | 2.11 | 0.63 |
| 52 | 0.40 | 1.16 | 4.57 | 6.4E-15 |
| 59 | 0.93 | 1.09 | 2.80 | 9.4E-05 |
| 56 | 0.17 | 2.45 | 1.32 | 0.01 |
| 68 | 1.02 | 0 | 0.82 | 0.74 |

| | PPV (%) by viral load | | | |
|---|---|---|---|---|
| | CIN3+ | | | |
| HPV type | Low | Intermediate | High | *P* value for trend[a] |
| *Any hrHPV*[b] | 0.98 | 1.90 | 4.66 | 5.3E-105 |
| 16 | 2.11 | 3.58 | 10.56 | 9.2E-56 |
| 33 | 1.00 | 6.20 | 3.93 | 0.01 |
| 31 | 1.75 | 4.32 | 1.77 | 0.05 |
| 18 | 1.25 | 0.95 | 2.52 | 0.01 |
| 35 | 0.85 | 1.97 | 2.50 | 0.01 |
| 58 | 0.22 | 0.29 | 2.20 | 1.5E-05 |
| 45 | 1.25 | 1.23 | 1.07 | 0.71 |
| 52 | 0.26 | 0.84 | 2.55 | 1.4E-09 |
| 59 | 0.42 | 0.65 | 1.51 | 8.7E-04 |
| 51 | 1.11 | 0.78 | 0.55 | 0.11 |
| 39 | 0.15 | 0.56 | 0.83 | 4.1E-03 |
| 56 | — | — | 0.45 | |
| 68 | — | — | 0.27 | |

NOTE: Color codes give categories of 3-year risk: CIN2+, <2%, 2%–5%, 5%–10%, and >10%; CIN3+, <1%, 1%–2%, 2%–5%, and >5%.

[a]*P* values for trend in PPV by increasing viral load category.

[b]Highest ranked hrHPV type per woman.



AACR American Association for Cancer Research
Cancer Epidemiology, Biomarkers & Prevention

BJC British Journal of Cancer www.nature.com/bjc

CONSENSUS STATEMENT

# Cervical screening: ESGO-EFC position paper of the European Society of Gynaecologic Oncology (ESGO) and the European Federation of Colposcopy (EFC)

Maria Kyrgiou[1,2], Marc Arbyn[3], Christine Bergeron[4], F. Xavier Bosch[5,6,7], Joakim Dillner[8], Mark Jit[9,10,11], Jane Kim[12], Mario Poljak[13], Pekka Nieminen[14], Peter Sasieni[15], Vesna Kesic[16], Jack Cuzick[17] and Murat Gultekin[18]

- **HR-HPV type-specific viral load** has been proposed as biomarker of risk.
- Some available HPV assays allow semi-quantitative viral load measuraments (relative light units for HC2 and CT scores for PCR-based tests).
- *Truly quantitative validated HPV tests are required allowing viral load adjustment for the number of cells per sample.*

# Biomarker molecolari per il TRIAGE di donne HPV-positive

## Trascritti oncogeni (E6/E7 mRNA) di HPV ad alto rischio

OXFORD

*JNCI J Natl Cancer Inst* (2021) 113(3): djaa105

doi: 10.1093/jnci/djaa105
First published online August 3, 2020
Article

### p16/ki67 and E6/E7 mRNA Accuracy and Prognostic Value in Triaging HPV DNA-Positive Women

Paolo Giorgi Rossi, PhD,[1,*] Francesca Carozzi, MSc,[2] Guglielmo Ronco, MD,[3,5] Elena Allia, MSc,[4] Simonetta Bisanzi, MSc,[2] Anna Gillio-Tos, PhD,[5] Laura De Marco, MSc,[4,5] Raffaella Rizzolo, BSc,[5] Daniela Gustinucci,[6] Annarosa Del Mistro,[7] Helena Frayle,[7] Massimo Confortini,[2] Anna Iossa,[8] Elena Cesarini,[6] Simonetta Bulletti, MSc,[6] Basilio Passamonti, MSc,[6] Silvia Gori,[7] Laura Toniolo, BSc,[9] Alessandra Barca, MSc,[10] Laura Bonvicini, BSc,[1] Pamela Mancuso, BSc,[1] Francesco Venturelli, MD,[1,11] Maria Benevolo, MSc,[12] and the New Technology for Cervical Cancer 2 Working Group

40 509 women were recruited, and 3147 (7.8%) tested HPV DNA positive; 174 CIN2+ with sensitivity of **61.0%** (95% confidence interval [CI] 1⁄4 53.6 to 68.0) for **cytology, 94.4%** (95% CI 1⁄4 89.1 to 97.3) for **E6/E7 mRNA, 75.2%** (95% CI 1⁄4 68.1 to 81.6) for **p16/ki67** was observed.

**Conclusions**: p16/ki67 showed good performance as a triage test. *E6/E7 mRNA showed the highest sensitivity, at the price of too high a positivity rate to be efficient for triage*. However, when negative, it showed a good prognostic value for clearance and CIN2þ regression.

# **VALTRIHP:**
# Validazione clinica di nuovi Test Molecolari nel TRIAGE di donne HPV-positive

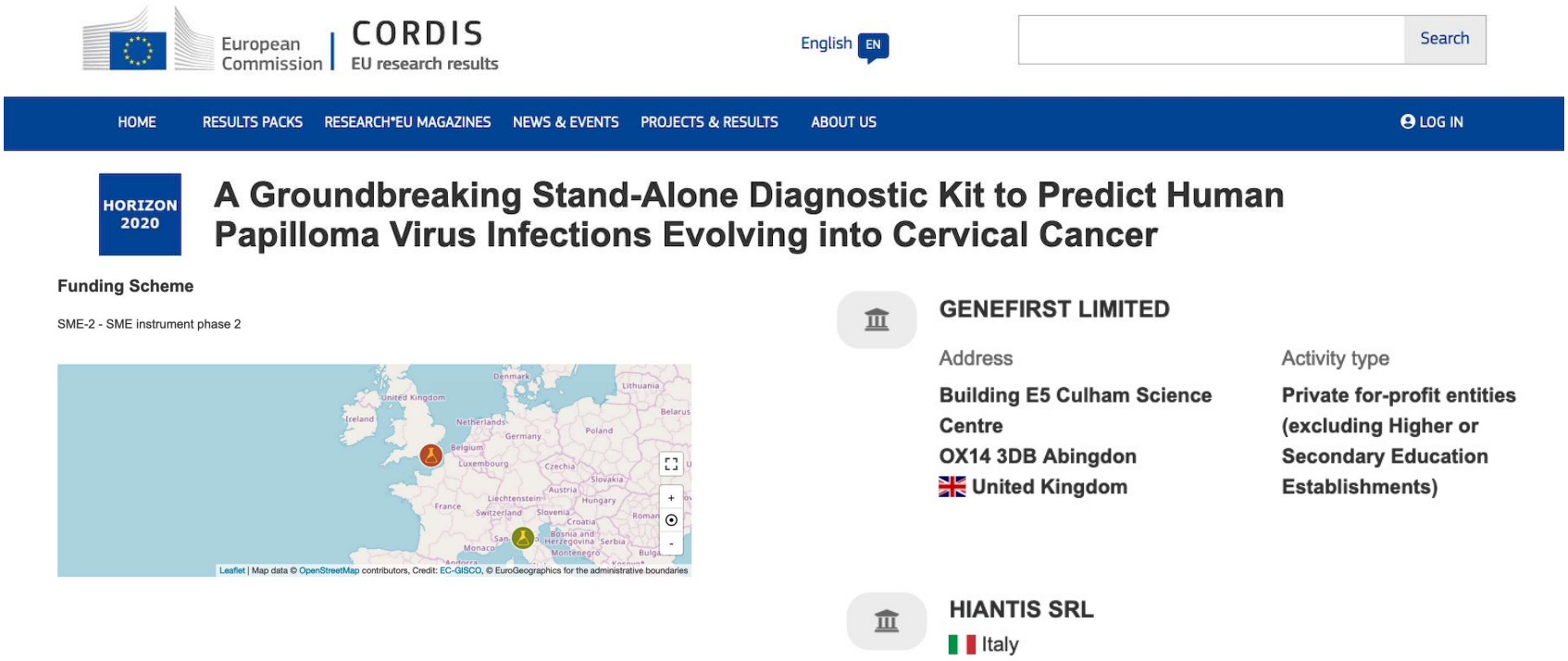
Clinical Performance Study Plan
(CPSP)

Study TITLE
**VALidation of tests usable as a first step TRIage to manage hrHPV Positive women (VALTRIHP)**

Study NUMBER: WP7-HPVONC
Study ACRONYM:VALTRIHP

**Main Project Scientific Coordinator**:
Marc Arbyn MD PhD
Unit of Cancer Epidemiology Belgian Cancer Centre
Sciensano, B-Brussels (Belgium)

**Coordinating Centre**:
SSD Epidemiologia e Screening – CRPT
A.O.U. Città della Salute e della Scienza di Torino
Corso Bramante, 88, 10126 Torino TO

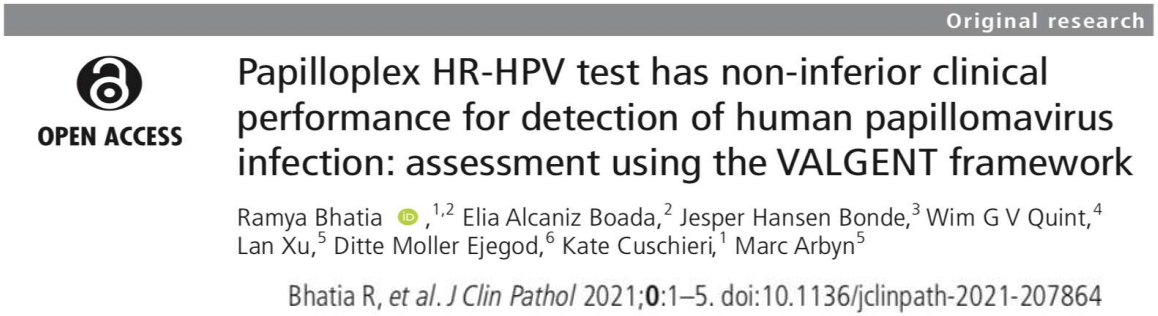



GENEFIRST Ltd è una piccola impresa inglese di recente costituzione (2011) con sede ad OXFORD

HIANTIS Srl è una piccola impresa italiana (MILANO) di recente costituzione (2014) con sede operativa a BRESCIA

# Biomarker molecolari per la Prevenzione del Carcinoma della Cervice Uterina

- **Tipizzazione completa dei 14 genotipi di HPV ad alto rischio**
  - **Papilloplex (GeneFirst)**



Original research

OPEN ACCESS

Papilloplex HR-HPV test has non-inferior clinical performance for detection of human papillomavirus infection: assessment using the VALGENT framework

Ramya Bhatia ,[1,2] Elia Alcaniz Boada,[2] Jesper Hansen Bonde,[3] Wim G V Quint,[4] Lan Xu,[5] Ditte Moller Ejegod,[6] Kate Cuschieri,[1] Marc Arbyn[5]

Bhatia R, *et al*. *J Clin Pathol* 2021;**0**:1–5. doi:10.1136/jclinpath-2021-207864

- **HR-HPV Screening & Quantificazione virale normalizzata**
  - **HPV OncoPredict (HIANTIS)**

- **Trascritti oncogeni**
  - **HPV OncoPredict (RNA) oncogenic transcripts (GeneFirst)**

# HPV OncoPredict:

## HR-HPV screening (SCR)

## &

## Quantificazione virale (QT) genotipo-specifica per 12 HR-HPV normalizzata

Abstract sottomesso ad EUROGIN 2022

**CLINICAL VALIDATION OF HPV OncoPredict® SCR AND QT ASSAYS USING THE VALGENT-2 FRAMEWORK**

Presenting author:
Sharonjit Kaur Dhillon
sharon.dhillon@sciensano.be
Unit of Cancer Epidemiology/Belgian Cancer Centre, Sciensano, Rue Juliette Wytsmanstraat 14, 1050 Brussels, Belgium

Sharonjit Kaur Dhillon[1], Marianna Martinelli[2], Chiara Giubbi[2], Kate Cuschieri[3], Clementina Cocuzza[2], and Marc Arbyn[1]

[1]Unit of Cancer Epidemiology/Belgian Cancer Centre, Sciensano, Brussels, Belgium.
[2]Department of Medicine and Surgery, University of Milano-Bicocca, Italy.
[3]Scottish HPV Reference Laboratory, Royal Infirmary of Edinburgh, Edinburgh, Scotland, United Kingdom.

**Background:** Assessment of emerging HPV assays for their use in cervical cancer screening is vital. HPV OncoPredict® SCR and QT (Hiantis Srl) are two independent multiplex real-time PCR assays targeting HR-HPV E6/E7 DNA comprising a "partial" genotyping screening assay (SCR) and a "full" genotyping, normalized viral load (QT) assay. OncoPredict® SCR identifies HPV16 and HPV18 separately and 11 other hrHPV types in aggregate while OncoPredict® QT detects 12 high risk (HR) HPV types independently. The assays can be used either alone or in combination, as a two-step reflex testing allowing HR-HPV genotype-specific viral load determination in screen-positive samples. Quality controls for sample adequacy, the efficiency of nucleic acid extraction and PCR inhibition are included in the testing.

**Objectives:** To evaluate the clinical performance of the HPV OncoPredict® SCR and QT using the international validation of HPV Genotyping Tests (VALGENT-2) framework.

**Methods:** The VALGENT-2 panel consists of 1,300 cervical liquid-based cytology (LBC) samples from women aged 20-60 attending the Scottish cervical cancer screening programme (1000 consecutive samples from routine screening enriched with 300 cytological abnormal samples). Disease was defined as histologically confirmed CIN2+ (n= 95 [denominator for sensitivity]), whereas two consecutive negative cytology results were accepted as a proxy for non-disease (n=721 [denominator for specificity]). Performance relative to GP5+/6+- PCR-EIA (standard comparator test) was assessed by a non-inferiority test.

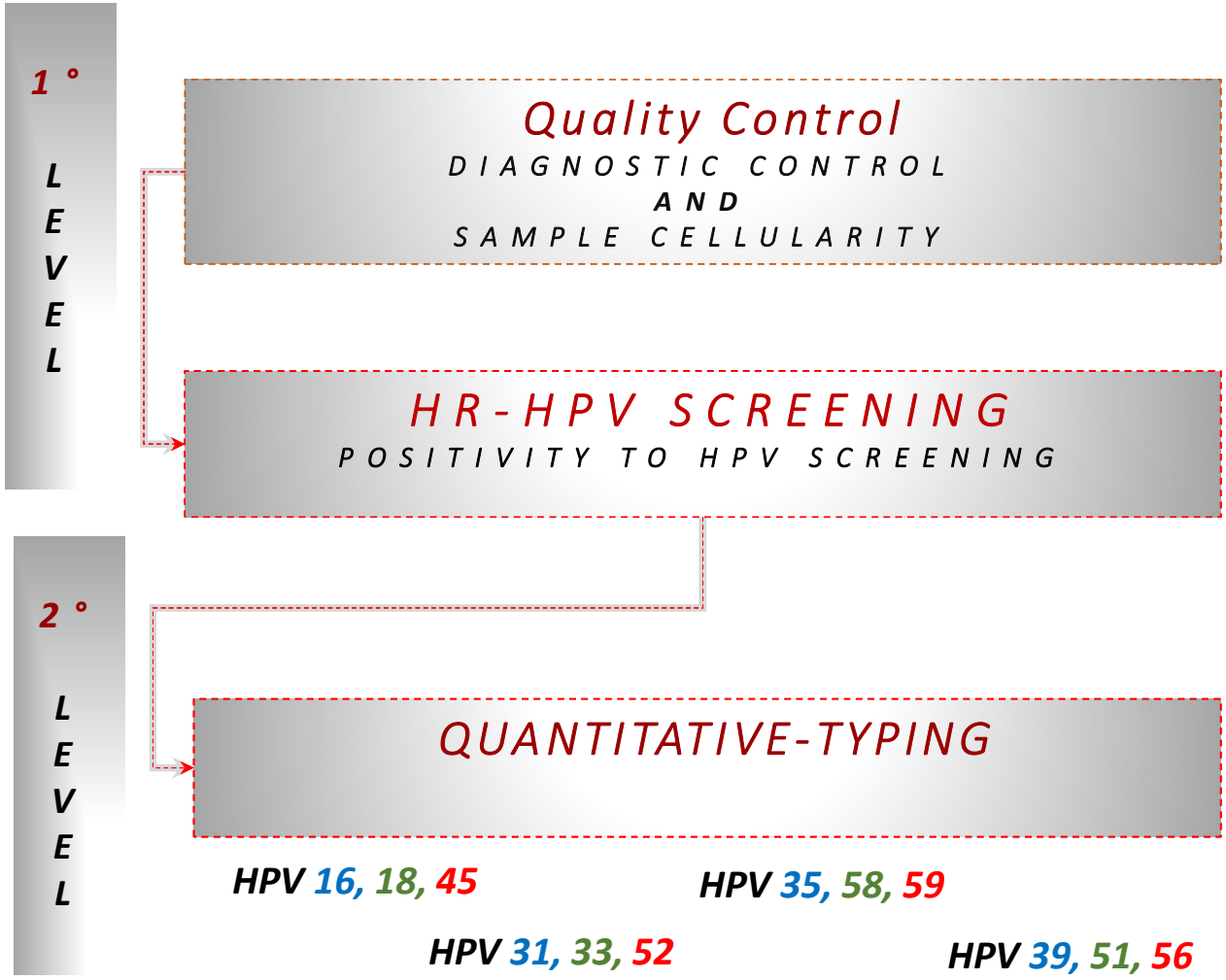
**Results:** The relative sensitivity and specificity for CIN2+ of OncoPredict® SCR vs GP5+/6+-PCR-EIA were 1.01 (95% CI, 0.99-1.03) and 1.02 (95% CI, 1.00-1.04). The results for OncoPredict® QT vs GP5+/6+-PCR-EIA were similar with a relative sensitivity of 1.01 (95% CI, 0.99-1.03) and relative specificity of 1.03 (95% CI, 1.0-1.06). The *p*-value for all the tests of non-inferiority was $p_{ni} \leq 0.001$. Both assays also showed non-inferior accuracy when restricted to women aged 30 years or older.

**Conclusions:** HPV OncoPredict® SCR and QT assays fulfil the clinical accuracy criteria for use in cervical cancer screening.

**Acknowledgments**: HPV OncoPredict® assays were developed as part of a European Commission funded SME Instrument Phase 2 Project (Grant agreement ID: 806551).

# Hiantis

# *HPV OncoPredict – Reflex Testing*




- ✓ ***AMPLIFICATION EFFICIENCY***
- ✓ *EXTRACTION EFFICIENCY*
- ✓ *SAMPLE ADEQUACY*

- ✓ *SELECT POSITIVE SAMPLES*

*LIMITED GENOTYPING:*
*HPV16 &HPV18 + 11 OTHER HR-HPV*

- ✓ ***FULL GENOTYPING:*** *12 HR-HPV*
  *GENOTYPE-SPECIFIC*
  *NORMALIZED VIRAL LOAD*

# Papilloplex High Risk Kits – Reflex Testing

**1° LIVELLO**

## HR-HPV DNA SCREENING
POSITIVITY TO HPV SCREENING

✓ *Genotype-specific qualitative assessment*

✓ *SELECT POSITIVE SAMPLES*
*14 hrHPV genotype specific DNA*

**2° LIVELLO**

## HR-HPV RNA TEST
Ricerca trascritti oncogeni virali

✓ *SELECT POSITIVE SAMPLES*
*14 hrHPV genotype specific RNA*

# VALTRIPH Study: Nuovi TEST

- ✓ **HPV OncoPredict SCR & QT (Hiantis)**: Limited Screening & 12 hrHPV normalized type-specific viral load
- ✓ **Papilloplex High Risk HPV (GeneFirst)**: 14 hrHPV type-specific DNA
- ✓ **Papilloplex mRNA (GeneFirst)**: 14 hrHPV type-specific mRNA

# IN VITRO DIAGNOSTIC MEDICAL DEVICE CLINICAL PERFORMANCE STUDY

**STUDY OBJECTIVE:**

**ALTERNATIVES TO CYTOLOGY for TRIAGE** of **HPV+ve women at Screening BASED** on **full genotyping** combined with **type-specific viral load (quantitative normalized viral DNA assays)** and/or **oncogenic transcripts (viral mRNA)**

**STUDY HYPOTHESIS:**

**Sensitivity and Specificity** of **markers/IVDs under investigation** NON INFERIOR **to current triage test (cytology)**

**DISEASE OUTCOME:**

**Histological diagnosis of CIN2+ and CIN3+** (CIN0 or CIN1 histology or colposcopy negative)

**STUDY DESIGN:**

- **Observational**
- **Cross-sectional** (follow up at one year of only HPV +ve, Cytology –ve with no further sample collection)

AZIENDA OSPEDALIERO - UNIVERSITARIA
Città della Salute e della Scienza di Torino

**WOMEN ATTENDING PRIMARY HPV-BASED CERVICAL CANCER SCREENING**

**AGE: 30-64**
**NO: HPV + in the previous year**
**NO: CIN + in the last 5 years**
**YES: Informed Consent**

**20.000 women**

**CERVICAL SAMPLE collection**

**ThinPrep**

**CERVICAL SAMPLING after 1 year**

**CERVICAL SAMPLING after 5 year**

**HPV -ve**

**HPV +ve**

**-**

**+**

**COLPOSCOPY HISTOLOGY**

**CYTOLOGY TRIAGE**

*digene* HC2 HPV DNA Test

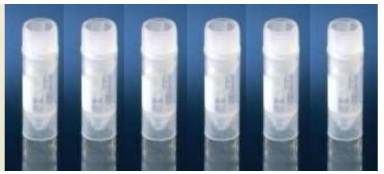
**2000 HPV +ve samples**

**GENOTYPING**

**DNA VIRAL LOAD**

**mRNA**

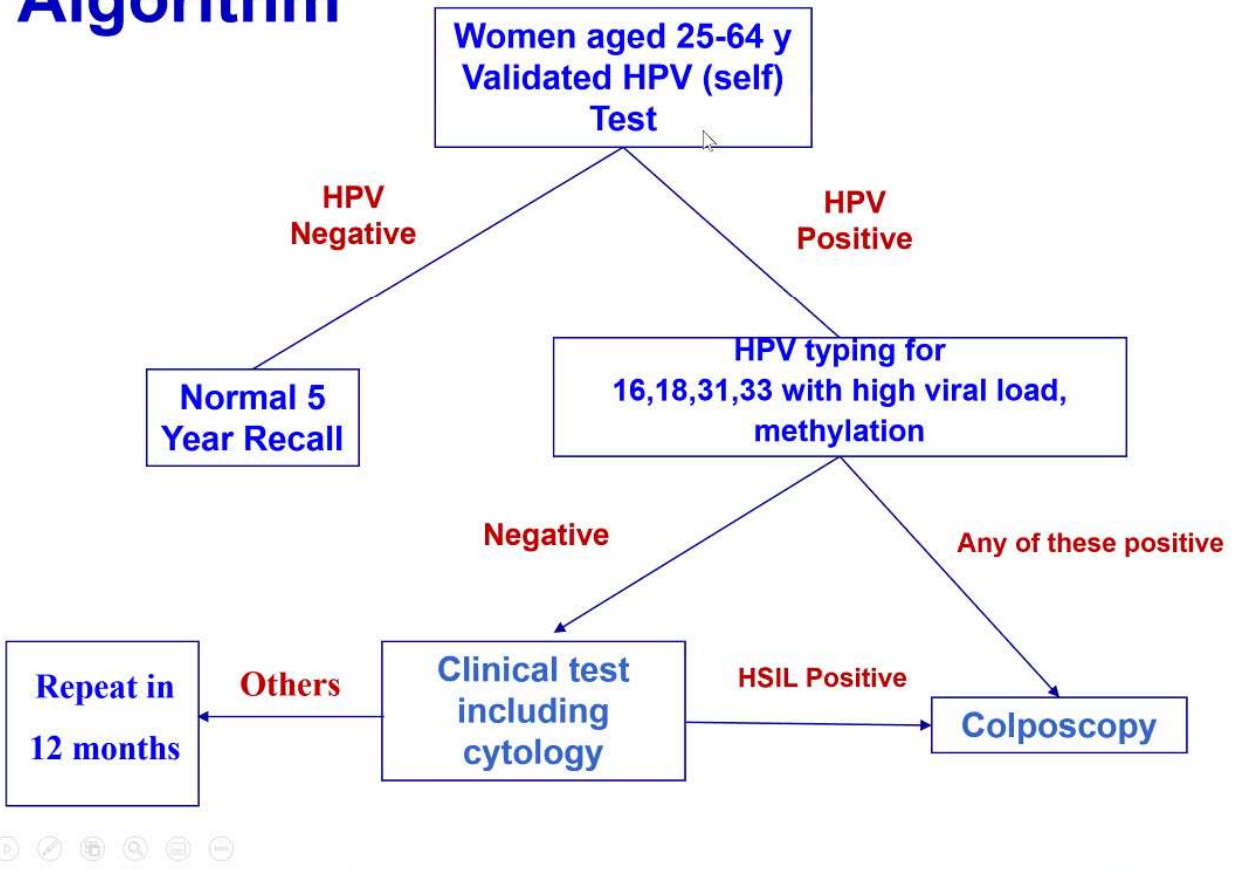

**FURTHER TESTING**



# VALTRIPH STUDY: TIMELINES

| FIRST SUBJECT ENROLLED | LAST SUBJECT EXPECTED | DELAYED TRIAGE COMPLETED |
| --- | --- | --- |
| 12.04.2021 | 30.03.2022 | 30.04.2023 |

# Potential Future Self-Screening Algorithm



# Ringraziamenti

AZIENDA OSPEDALIERO - UNIVERSITARIA
Città della Salute e della Scienza di Torino

sciensano

***CPO Torino***
Livia Giordano
Paola Armaroli
Raffaella Rizzolo
Elena Pizzotti

**Laboratorio Molecolare HPV**
**S.C. Anatomia Patologica 1U - SS Screening per il cervicocarcinoma (Resp.: Luigia Macrì)**
Laura De Marco
Marco Peradotto

**Marc Arbyn**
***Coordinator Belgian Cancer Centre - Sciensano***

***Ostetriche – Regione Piemonte***

***Laboratorio di Microbiologia e Virologia Clinica***
***Università di Milano-Bicocca***
Marianna Martinelli
Chiara Giubbi

UNIVERSITÀ DEGLI STUDI DI MILANO BICOCCA

# Ringraziamenti

Funding provided by the European Commission as part of a Horizon 2020 SME Instrument Phase 2 Project: HPV OncoPredict - Grant ID: 806551


# Grazie per l’attenzione!