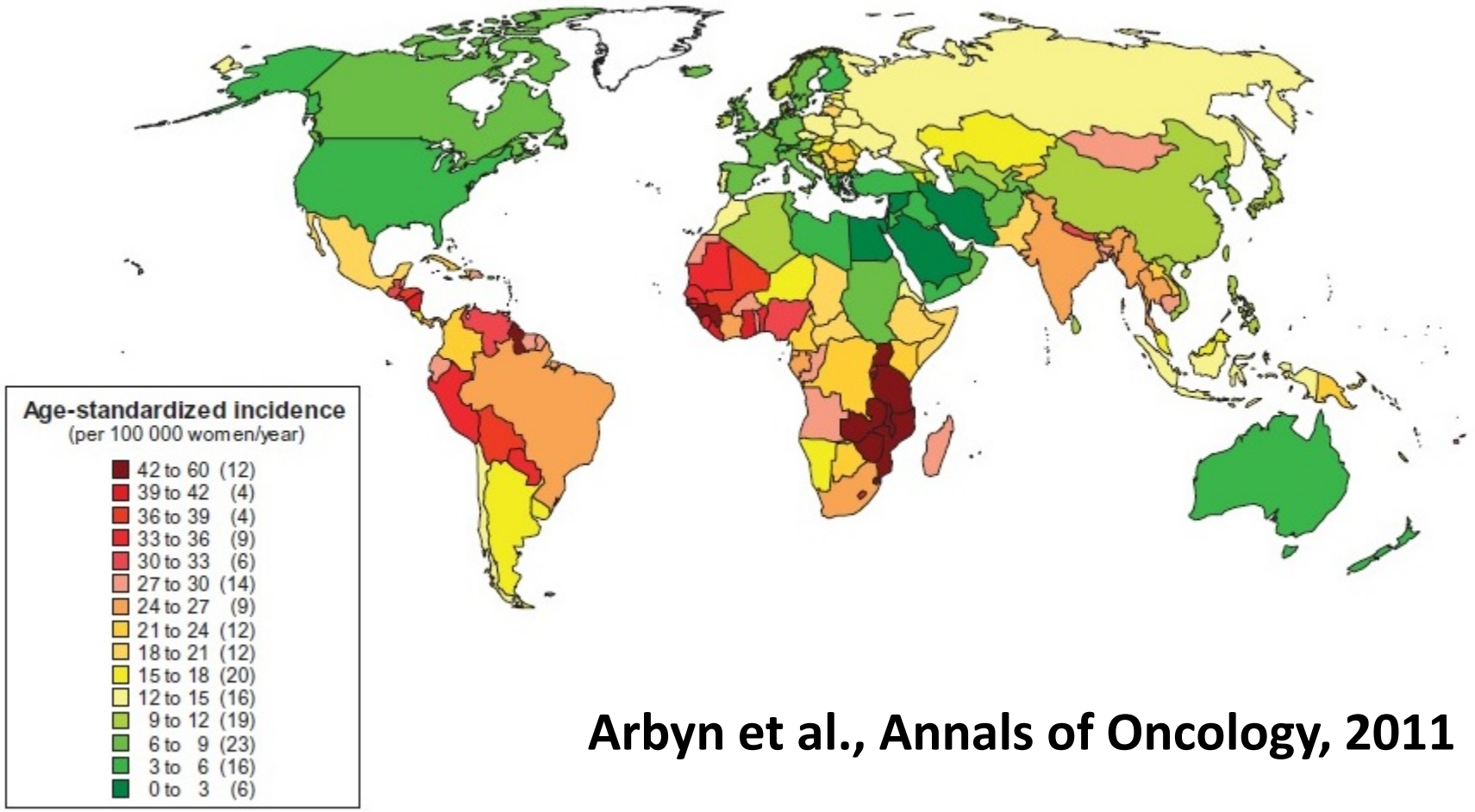
# ΤΙ ΝΕΟΤΕΡΟ ΣΤΗΝ ΠΡΟΛΗΨΗ ΤΟΥ ΚΑΡΚΙΝΟΥ ΤΡΑΧΗΛΟΥ ΜΗΤΡΑΣ

## Εναλλακτικές τεχνικές - δευτερογενής πρόληψη σε HPV test

Χριστίνα Κοτταρίδη, Βιολόγος MSc, PhD

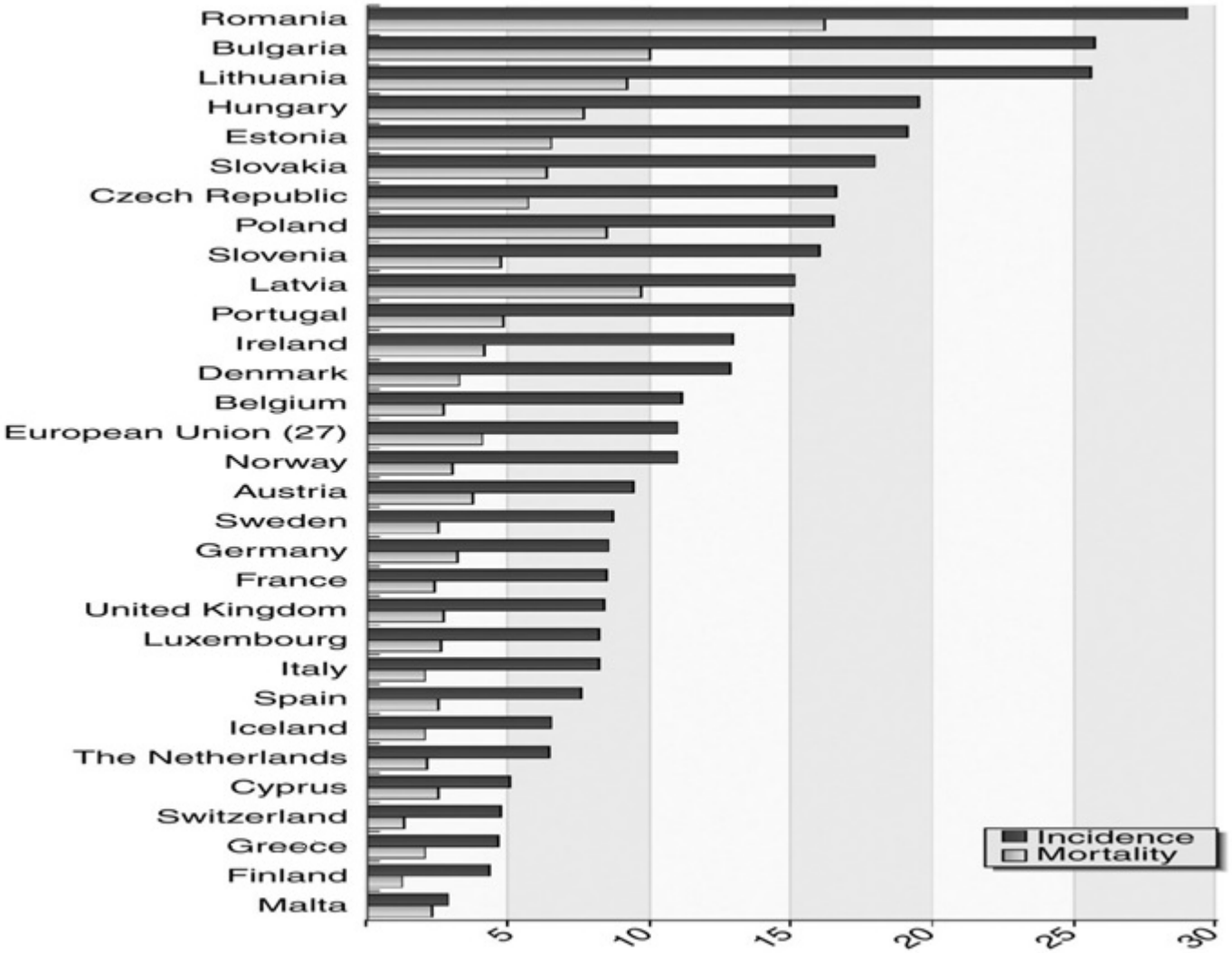
20-10-2014

# Παγκόσμια γεωγραφική κατανομή της επίπτωσης του καρκίνου του τραχήλου της μήτρας




**Arbyn et al., Annals of Oncology, 2011**

# Εκτιμούμενη επίπτωση και θνησιμότητα από καρκίνο του τραχήλου της μήτρας στην Ευρώπη




Kesic V et al. Cancer Epidemiol Biomarkers Prev 2012;21:1423-1433

Cancer Epidemiology, Biomarkers & Prevention

# Στόχος

- Μείωση της επίπτωσης του καρκίνου του τραχήλου της μήτρας

Μόνο το τεστ Παπανικολάου αρκεί;

# Αιτιολογικός παράγοντας

- Εμμένουσα λοίμωξη από υψηλού κινδύνου ιό HPV

- Stefania Jablonska - Poland, 1972, epidermodysplasia verruciformis
- Jablonska και Gerard Orth ανακάλυψαν τον HPV-5 στον καρκίνο του δέρματος, 1978
- Harald zur Hausen και συν. 1983, ταυτοποίησαν τον HPV 16 και HPV 18 στον καρκίνο του τραχήλου της μήτρας
- Harald zur Hausen, 2008, Nobel Prize της Ιατρικής

# Ανίχνευση του ιού

- Liquid Based Cytology
- Ανίχνευση και ενίσχυση του ιικού DNA
- Ανίχνευση του mRNA των ιικών ογκογονιδίων E6 και E7

# Εκχύλιση γενετικού υλικού


# DNA

- Μέθοδοι που βασίζονται στο PCR
- Μέθοδοι που βασίζονται στο real-time PCR
- Μέθοδοι που δε βασίζονται στο PCR

## mRNA E6 και E7 ογκογονιδίων του ιού

JCM
Journals.ASM.org

# Global Improvement in Genotyping of Human Papillomavirus DNA: the 2011 HPV LabNet International Proficiency Study

**Carina Eklund,[a,b] Ola Forslund,[a] Keng-Ling Wallin,[c] Joakim Dillner[a,b]**

WHO HPV LabNet Global Reference Laboratory, Departments of Clinical and Medical Microbiology, Laboratory Medicine Skåne and Lund University, Malmö, Sweden[a]; Departments of Laboratory Medicine, Medical Epidemiology & Biostatistics, Karolinska Institute and Hospital, Stockholm, Sweden[b]; Equalis AB, Uppsala, Sweden[c]

Accurate and internationally comparable human papillomavirus (HPV) DNA genotyping is essential for HPV vaccine research and for HPV surveillance. The HPV Laboratory Network (LabNet) has designed international proficiency studies that can be issued regularly and in a reproducible manner. The 2011 HPV genotyping proficiency panel contained 43 coded samples composed of purified plasmids of 16 HPV types (HPV6, -11, -16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, -68a, and -68b) and 3 extraction controls. Tests that detected 50 IU of HPV16 and HPV18 and 500 genome equivalents for the other 14 HPV types in both single and multiple infections were considered proficient. Ninety-six laboratories worldwide submitted 134 data sets. Twenty-five different HPV genotyping assay methods were used, including the Linear Array, line blot/INNO-LiPA, Papillo-Check, and PCR Luminex assays. The major oncogenic HPV types, HPV16 and HPV18, were proficiently detected in 97.0% (113/116) and 87.0% (103/118) of the data sets, respectively. In 2011, 51 data sets (39%) were 100% proficient for the detection of at least one HPV type, and 37 data sets (28%) were proficient for all 16 HPV types; this was an improvement over the panel results from the 2008 and 2010 studies, when <25 data sets (23% and 19% for 2008 and 2010, respectively) were fully proficient. The improvement was also evident for the 54 laboratories that had also participated in the previous proficiency studies. In conclusion, a continuing global proficiency program has documented worldwide improvement in the comparability and reliability of HPV genotyping assay performances.

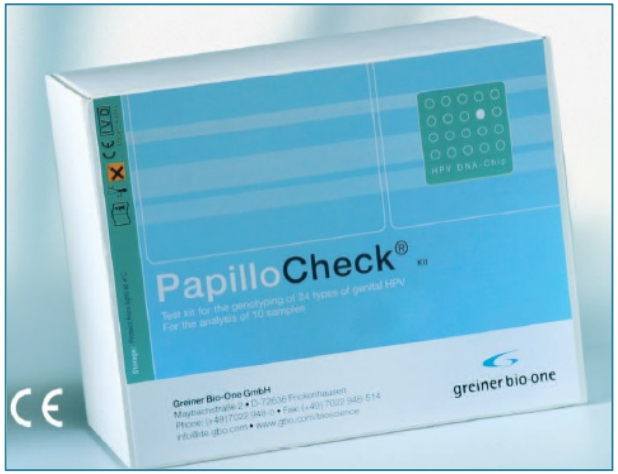
**TABLE 2** Proficiency of detecting HPV types tested for by assay type[a]

| HPV assay type (manufacturer) | No. of data sets | HPV region(s) targeted (primers) | No. of data sets with a proficiency (%) of: 100 | 90–99 | 80–89 | <80 | Not proficie |
|---|---|---|---|---|---|---|---|
| Linear Array (Roche)[b] | 18 | L1 (PGMY) | 13 | 0 | 1 | 1 | 4 |
| In-house PCR Luminex[c] | 11 | E6, E7, L1 | 5 | 3 | 1 | 0 | 2 |
| PapilloCheck (Greiner) | 9 | E1 | 2 | 3 | 3 | 1 | 0 |
| INNO-LiPA (Innogenetics) | 8 | L1 (SPF10) | 0 | 0 | 2 | 0 | 6 |
| In-house line blot[d] | 8 | E6, E7, L1 | 1 | 1 | 4 | 0 | 2 |
| In-house PGMY-CHUV[b] | 8 | L1 (PGMY) | 6 | 0 | 0 | 1 | 1 |
| In-house real-time PCR[e] | 7 | L1/E1/E4/E6/E7 | 5 | 1 | 0 | 0 | 1 |
| In-house type-specific PCR[f] | 7 | L1/L2/E1/E5/E6/E7/URR | 1 | 1 | 1 | 1 | 3 |
| CLART HPV 2/3 (Genomica)[b] | 6 | L1 (PGMY) | 0 | 0 | 2 | 2 | 2 |
| Hybribio 21 HPV GenoArray | 5 | L1 (PGMY) | 3 | 0 | 2 | 0 | 0 |
| Cobas 4800 (Roche)[g] | 4 | L1 | 2 | 0 | 1 | 1 | 0 |
| In-house PCR-EIA[h] | 4 | L1/E6/E7 | 3 | 0 | 0 | 0 | 1 |
| In-house PCR-RFLP[i] | 3 | L1/E6/E7 | 0 | 0 | 0 | 2 | 1 |
| In-house pyrosequencing[j] | 3 | L1 (GP/PGMY) | 0 | 0 | 1 | 1 | 1 |
| Hybribio 14 HR[g] | 3 | E6/E7 | 3 | 0 | 0 | 0 | 0 |
| Luminex PCR (Multimetrix) | 2 | L1 (GP) | 0 | 1 | 1 | 0 | 0 |
| In-house microarray chip[b] | 2 | L1 (My/GPM) | 1 | 0 | 0 | 0 | 1 |
| PANArray | 2 | L1 (GP) | 0 | 2 | 0 | 0 | 0 |
| Digene HPV genotyping RH test (Qiagen)[k] | 2 | L1 (GP) | 0 | 0 | 1 | 0 | 1 |
| Hybribio 13 HR[l] | 2 | E6/E7 | 2 | 0 | 0 | 0 | 0 |
| HPV Direct-Flow chip (Master Diagnóstica) | 2 | L1 (GP) | 2 | 0 | 0 | 0 | 0 |
| SPF(10)-LiPA25 version 1 (Labo Biomedical Products) | 2 | L1 (SPF10) | 0 | 1 | 0 | 0 | 1 |
| LCD array (Chipron)[b] | 2 | L1 (PGMY) | 2 | 0 | 0 | 0 | 0 |
| Other commercial assays[m] | 10 | L1/E1/E2/E6/E7 | 3 | 2 | 0 | 1 | 4 |
| Other in-house assays[n] | 4 | L1/E1/E7 | 0 | 0 | 0 | 2 | 2 |

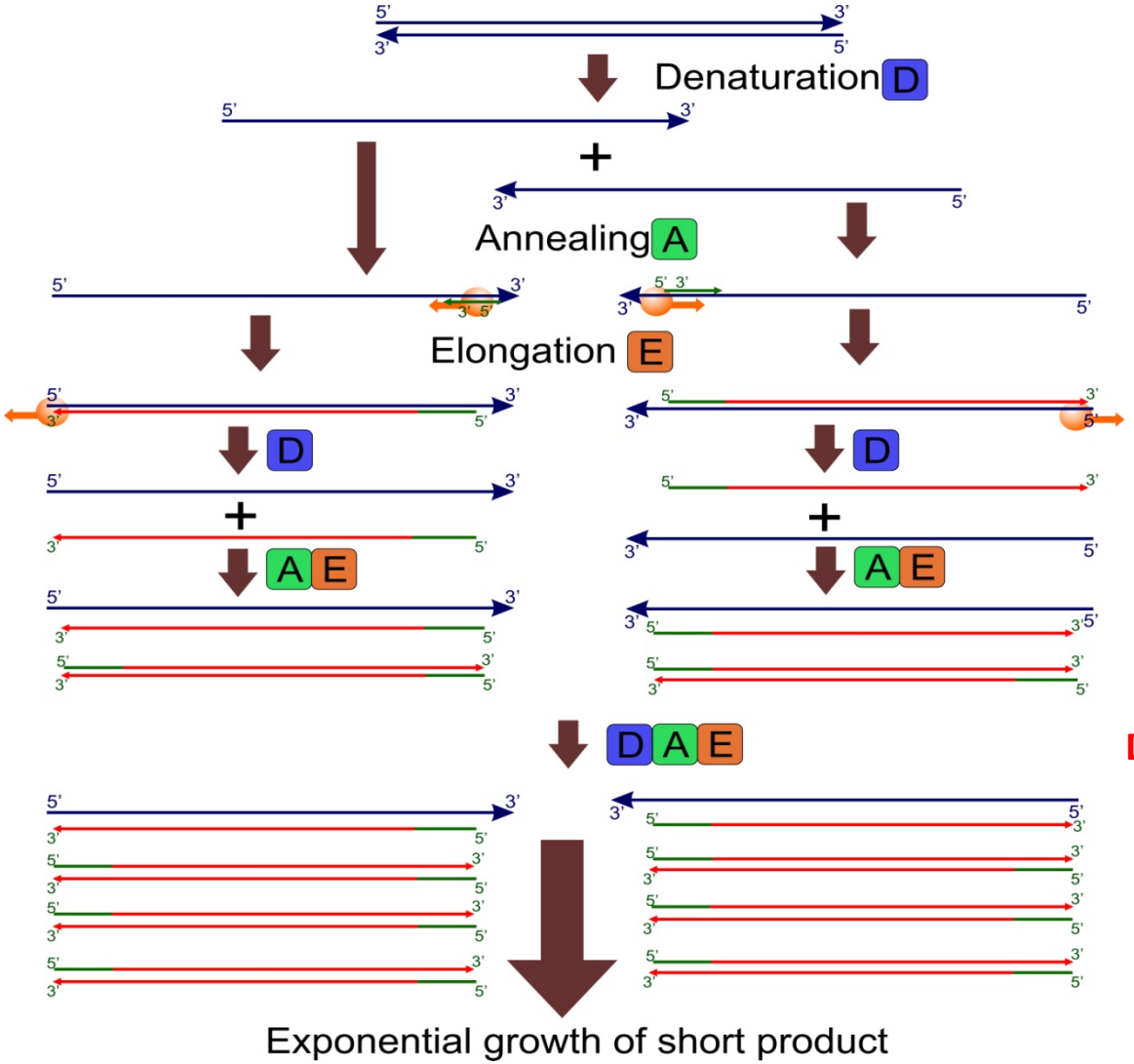
# Μέθοδοι που βασίζονται στο PCR

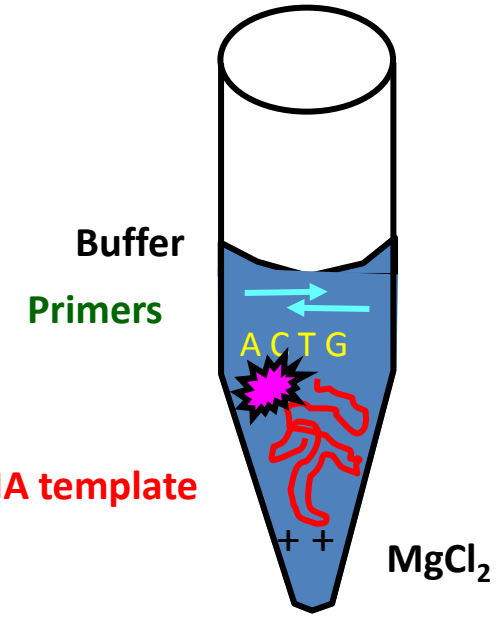
- AMPLICOR® - LINEAR ARRAY HPV GENOTYPING Tests
- CLART® HPV2
- INNO-LiPa HPV
- HPV Direct Flow-chip
- PapilloCheck® HPV-Screening
- Hybribio 21 HPV GenoArray
- GenoFlow HPV array (DiagCor)





# PCR




$n^{36}$ = 68,719,476,736 copies in ~ 2 hrs

16

18 26

31 33 35

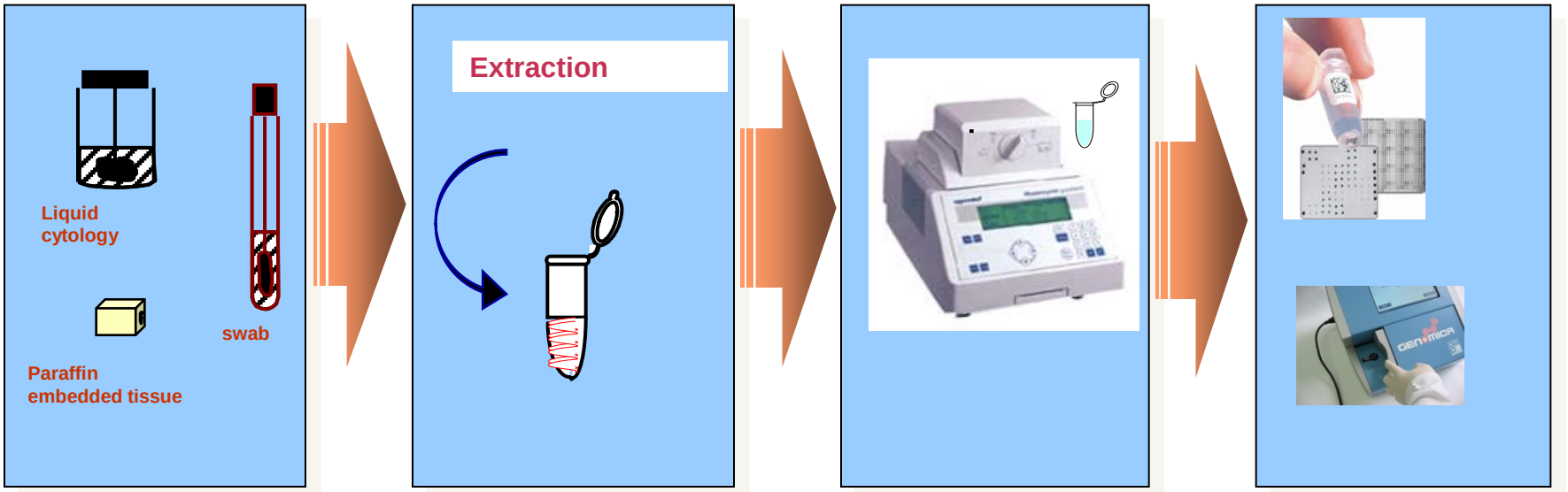
39 45 51 52

53 56 58 59 66

68 70 73 82 85

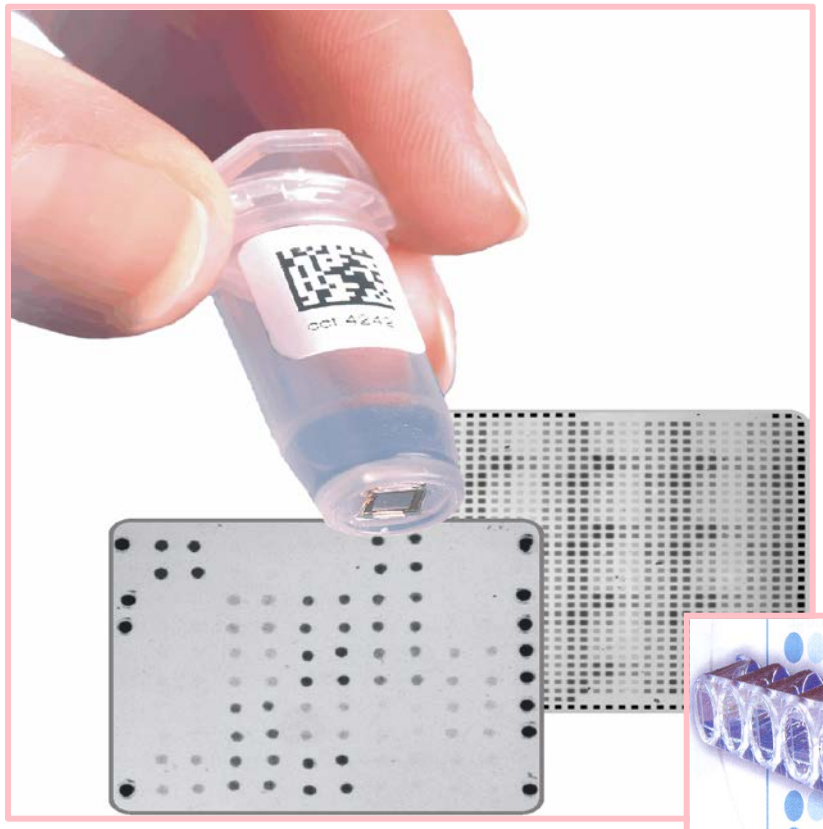
6 11 40 42 43 44 54

61 62 71 72 81 83 84 89




# HPV CLINICAL MICROARRAYS

# Microarrays Technology

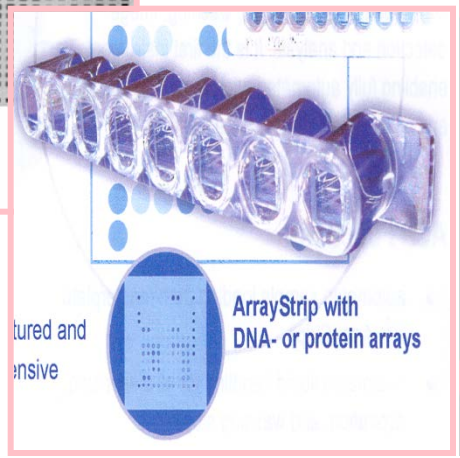


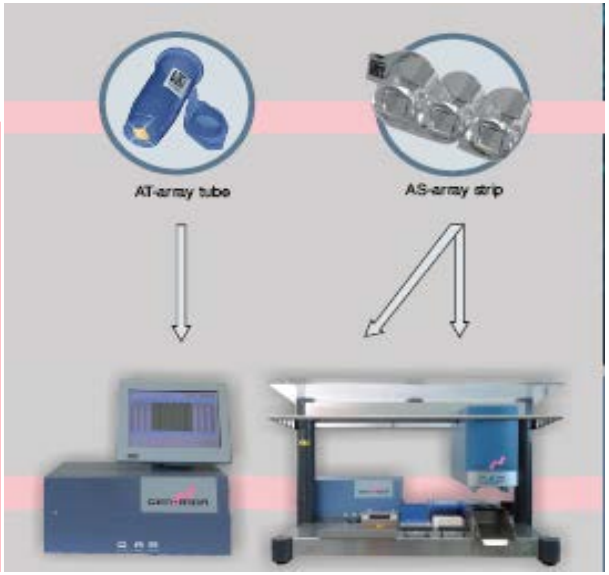
**Biochip** (3x3mm):

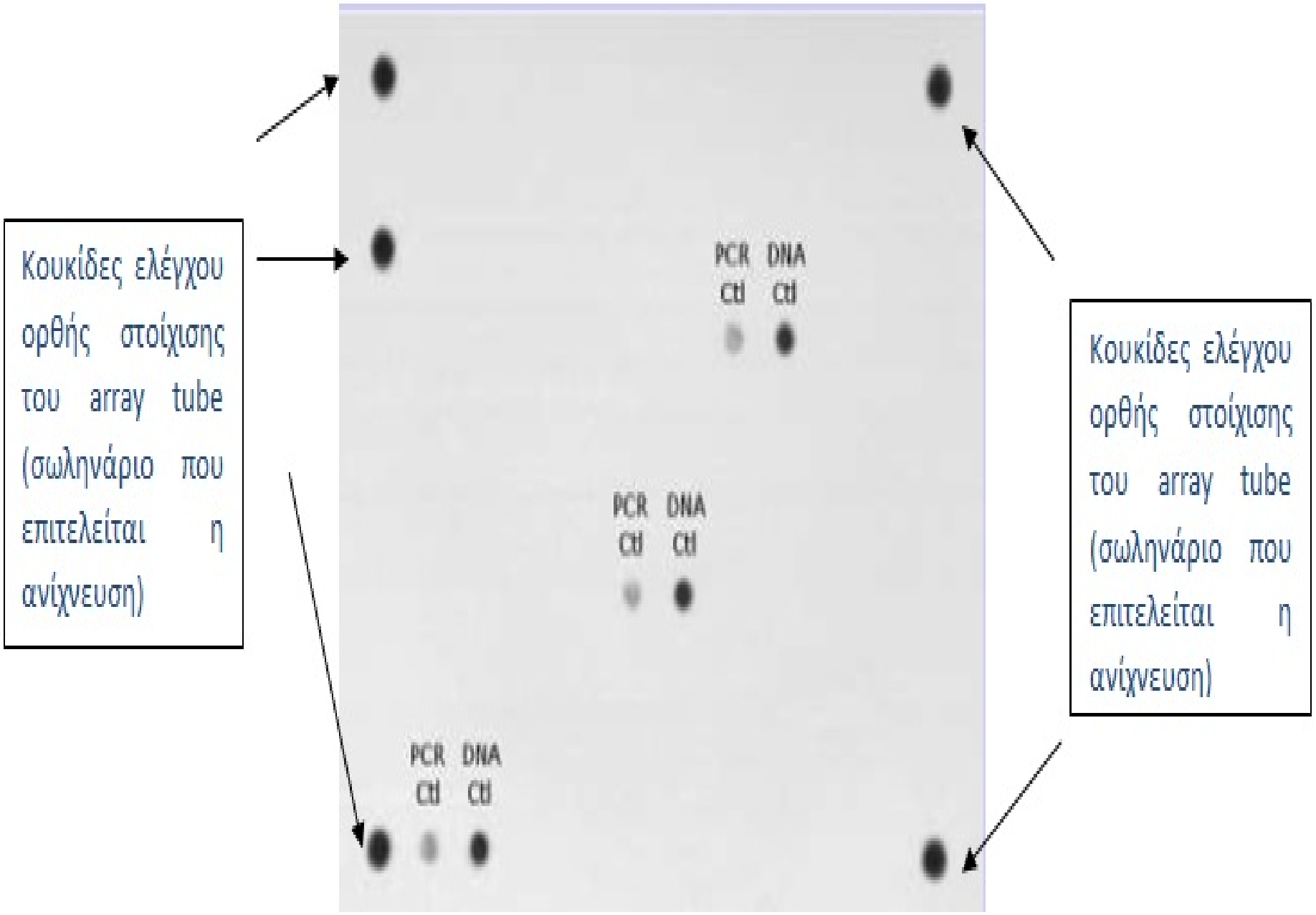
probes in triplicate

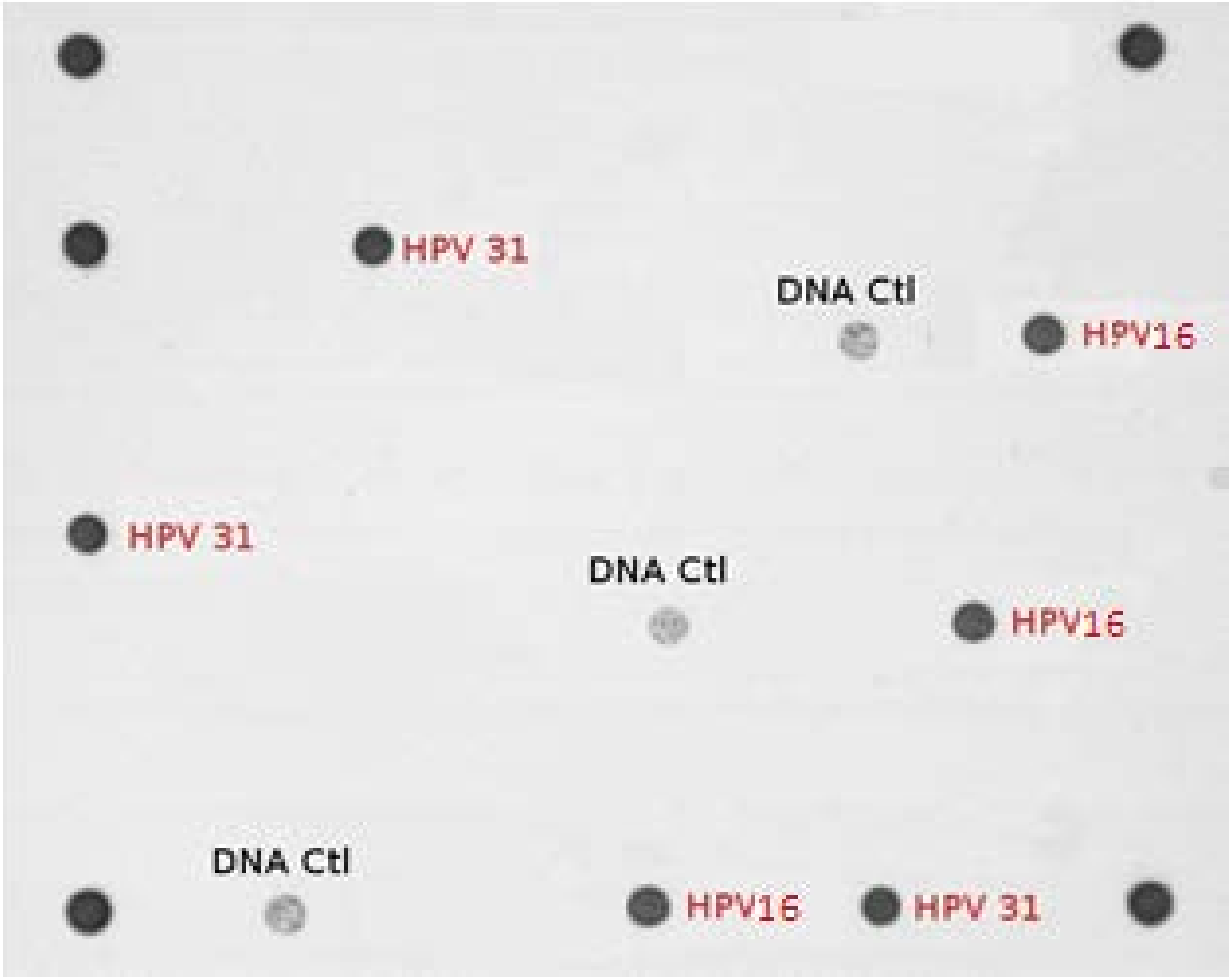
Internal control for nucleic acid extraction

Internal control for PCR inhibition

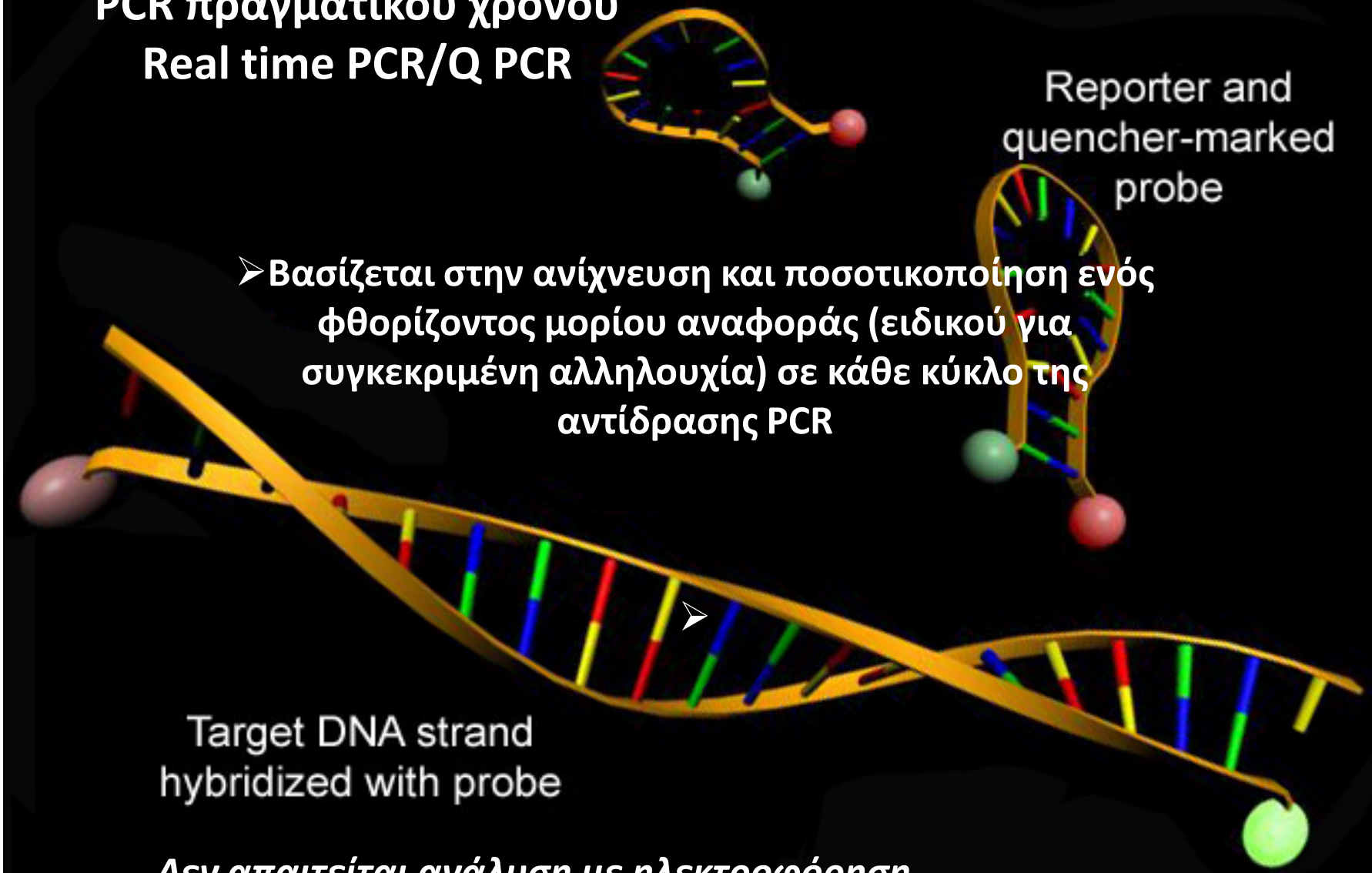



Κουκίδες ελέγχου ορθής στοίχισης του array tube (σωληνάριο που επιτελείται η ανίχνευση)
PCR Ctl
DNA Ctl
PCR Ctl
DNA Ctl
PCR Ctl
DNA Ctl
Κουκίδες ελέγχου ορθής στοίχισης του array tube (σωληνάριο που επιτελείται η ανίχνευση)


HPV 31
DNA Ctl
HPV16
HPV 31
DNA Ctl
HPV16
DNA Ctl
HPV16
HPV 31

# PCR πραγματικού χρόνου
# Real time PCR/Q PCR



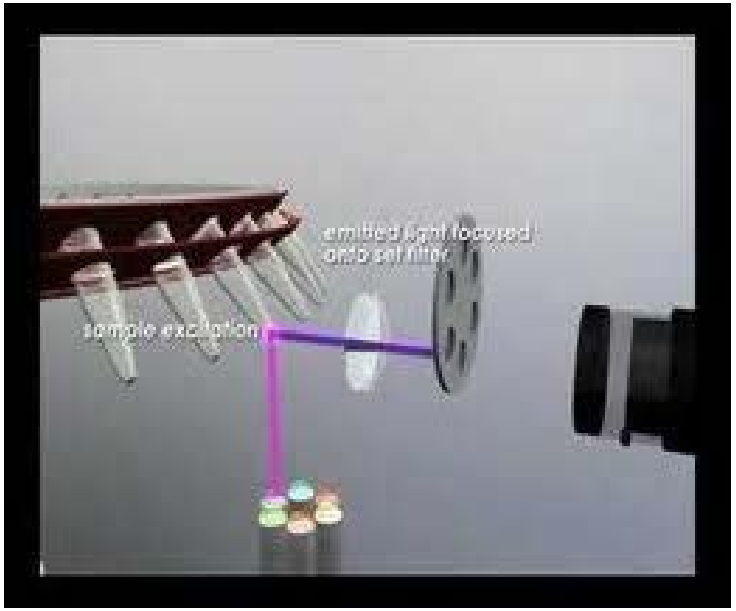

- **Βασίζεται στην ανίχνευση και ποσοτικοποίηση ενός φθορίζοντος μορίου αναφοράς (ειδικού για συγκεκριμένη αλληλουχία) σε κάθε κύκλο της αντίδρασης PCR**

***Δεν απαιτείται ανάλυση με ηλεκτροφόρηση***

# PCR πραγματικού χρόνου (qPCR)

➢ **Επιτρέπει**

✓την αδιάλειπτη παρακολούθηση των προϊόντων της PCR κατά την ενίσχυση μέσω της μέτρησης του εκπεμπόμενου φθορισμού των προϊόντων σε κάθε κύκλο

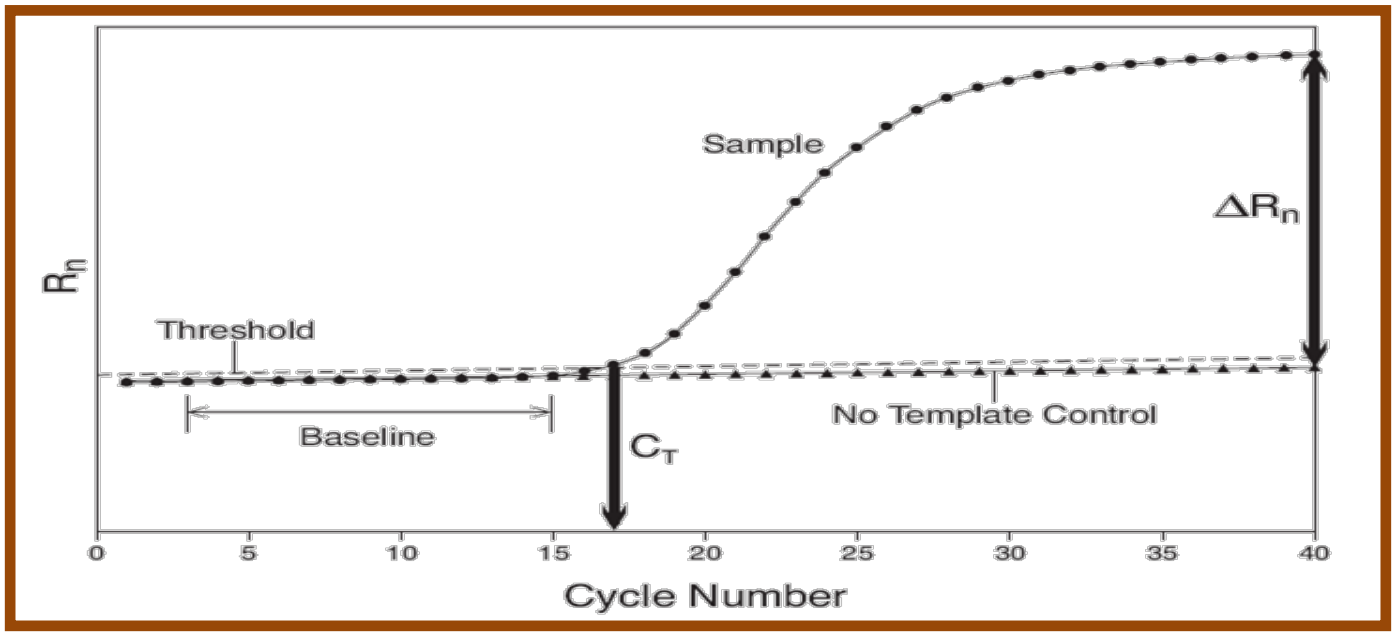


# PCR πραγματικού χρόνου (qPCR)

## ➢Επιτρέπει

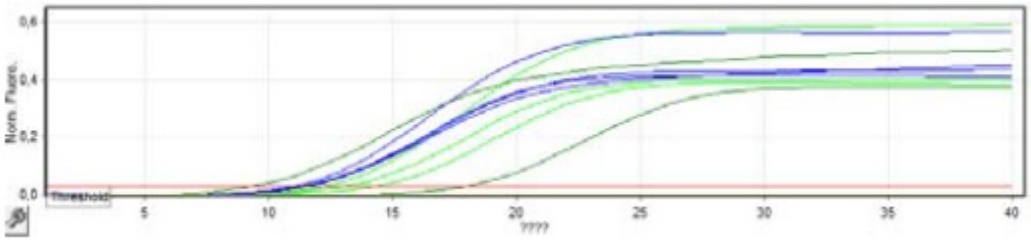
✓Ταυτοποίηση του κύκλου εισόδου στην εκθετική φάση (κύκλος-κατώφλι) και, της σχετικής ποσοτικοποίησης της μήτρας DNA στην αρχή της αντίδρασης.

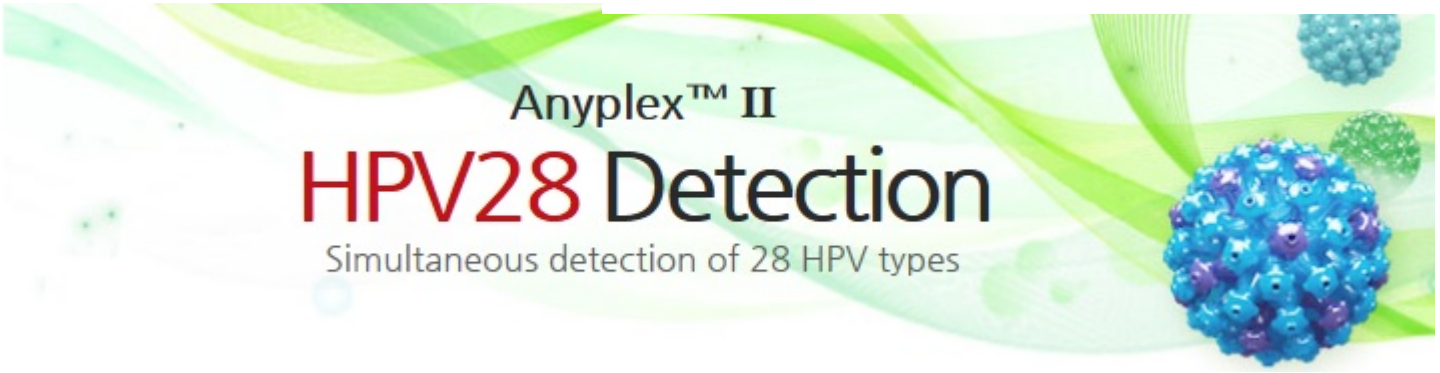
✓Τα προϊόντα του πολλαπλασιαμού αναλύονται σε πραγματικό χρόνο όπως συντίθενται σε κάθε κύκλο.



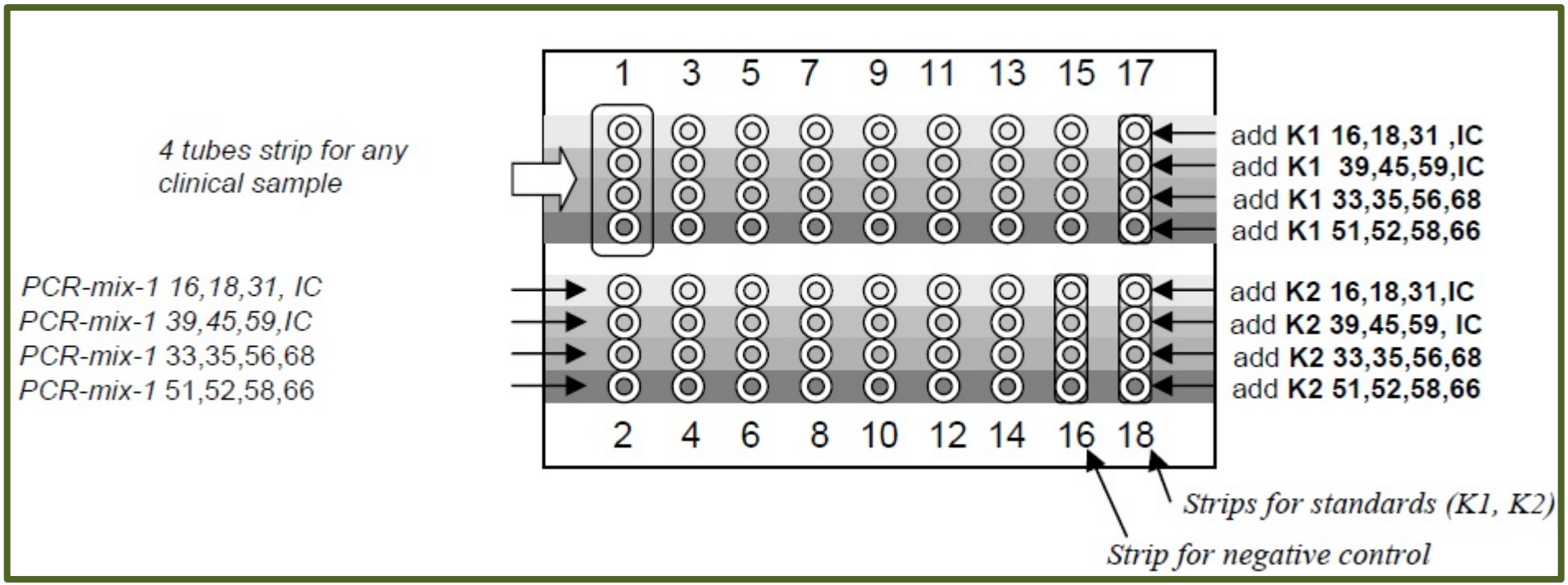
# Μέθοδοι που βασίζονται σε PCR πραγματικού χρόνου

- cobas® HPV Test
- Anyplex™ II HPV28 Detection
- Abbott RealTime High HPV
- Sacace 14 HR

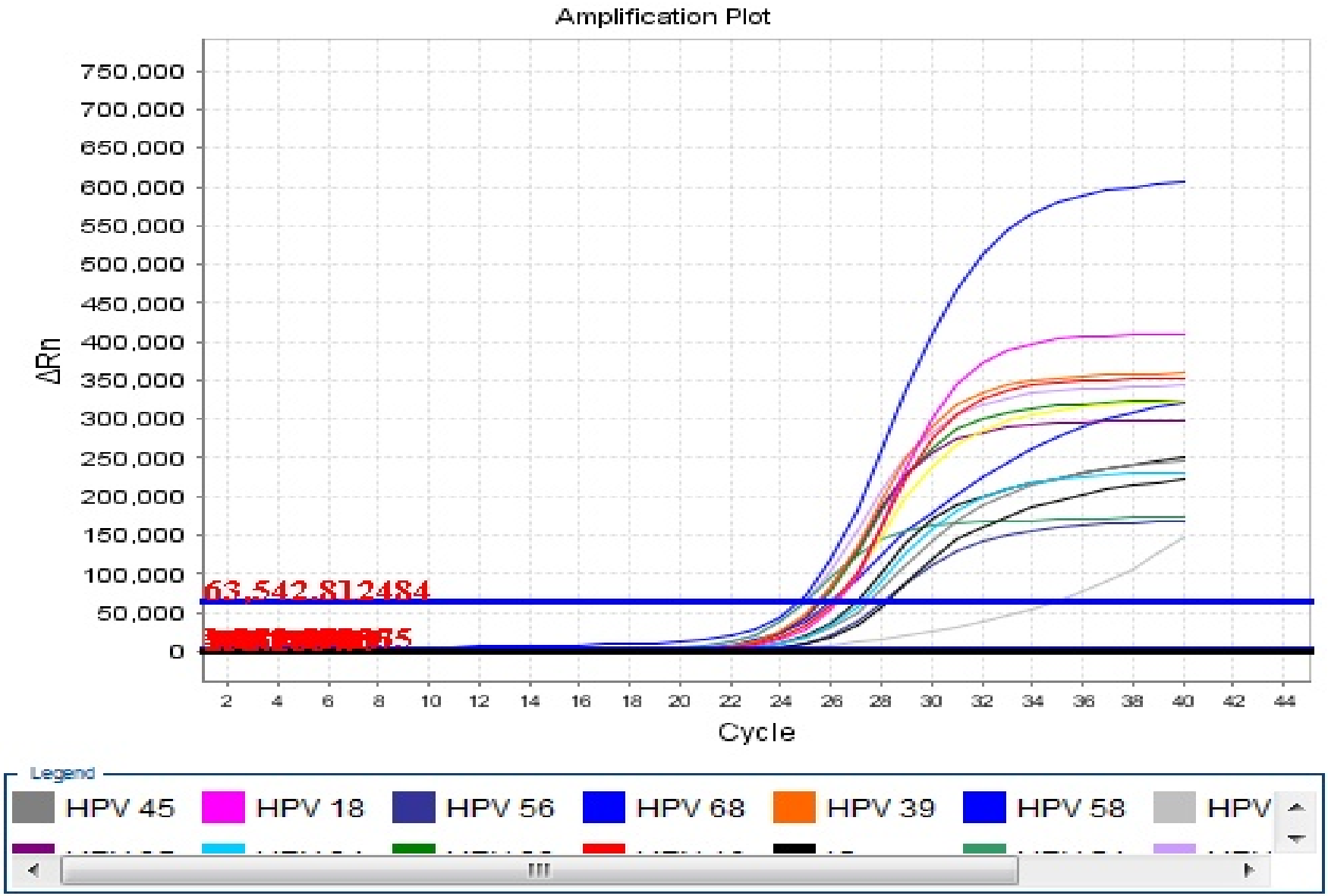
# HPV Genotypes 14 Real-TM Quant



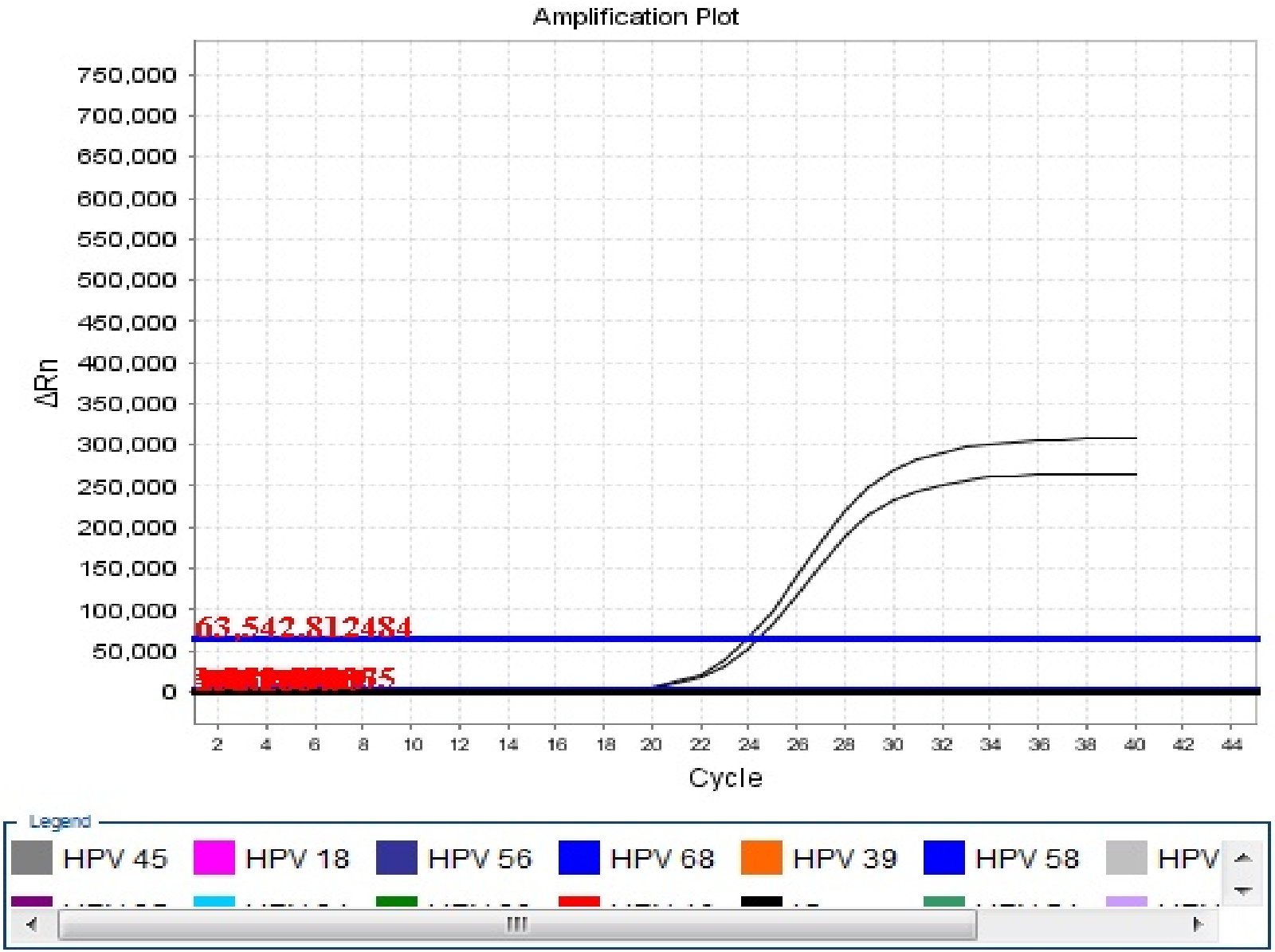

| FAM | JOE | ROX | Cy5 |
|---|---|---|---|
| 16 | 31 | 18 | IC |
| 39 | 45 | 59 | IC |
| 33 | 35 | 68 | 56 |
| 58 | 52 | 66 | 51 |

**For quantitative detection and genotyping of Human Papillomavirus (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68)**

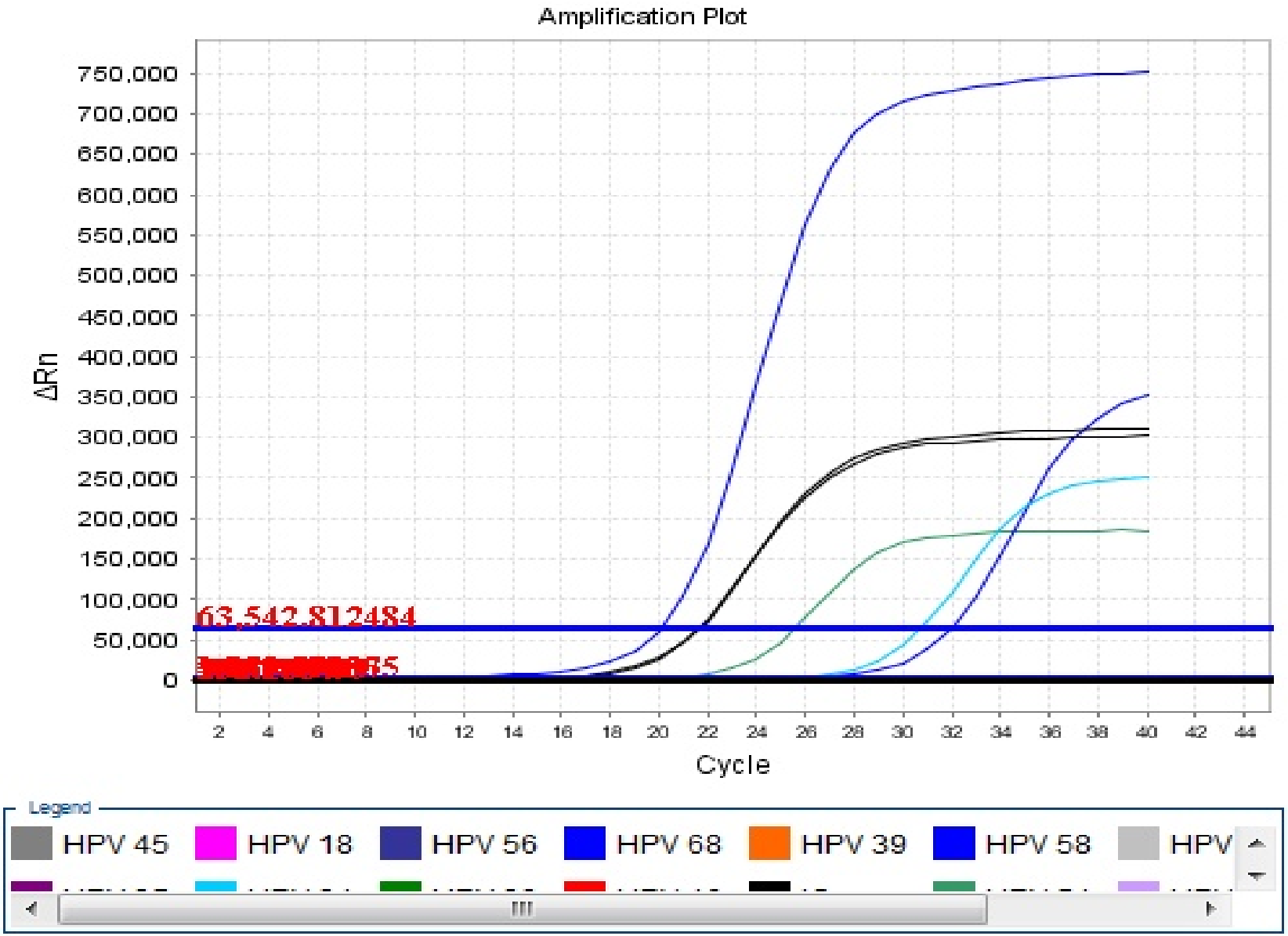
# Positive control



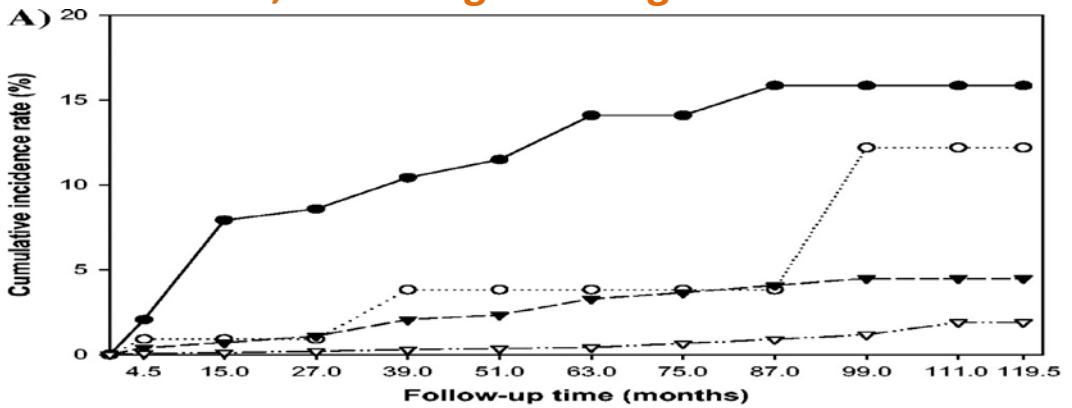
# Negative clinical sample



# Positive clinical sample

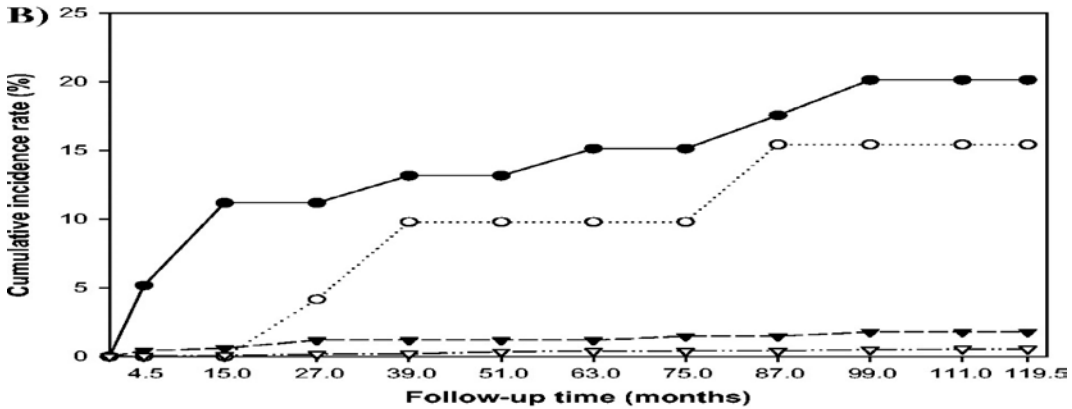


# Cumulative incidence of cervical intraepithelial neoplasia grade 3 and cancer (≥CIN3) over a 10-year period in A) 7285 women younger than 30 years of age and B) 13 229 women 30 years old and older, according to oncogenic HPV status at enrollment




No. of women seen during follow-up interval

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| HPV16+ | 339 | 184 | 140 | 99 | 84 | 68 | 61 | 49 | 57 | 21 | 1 |
| HPV18+ | 110 | 62 | 50 | 34 | 26 | 26 | 26 | 21 | 23 | 13 | 1 |
| HC2+ | 1249 | 663 | 514 | 407 | 352 | 312 | 261 | 228 | 229 | 112 | 7 |
| HC2- | 5498 | 2896 | 2349 | 1957 | 1695 | 1493 | 1285 | 1214 | 1083 | 543 | 23 |




No. of women seen during follow-up interval

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| HPV16+ | 116 | 63 | 50 | 45 | 41 | 44 | 33 | 35 | 32 | 14 | 2 |
| HPV18+ | 44 | 23 | 24 | 17 | 17 | 15 | 10 | 16 | 12 | 3 | 0 |
| HC2+ | 962 | 545 | 502 | 455 | 403 | 389 | 339 | 300 | 318 | 144 | 10 |
| HC2- | 11893 | 6863 | 6323 | 5856 | 5441 | 4986 | 4675 | 4337 | 4195 | 2078 | 133 |

**Khan M J et al. JNCI J Natl Cancer Inst 2005;97:1072-1079**



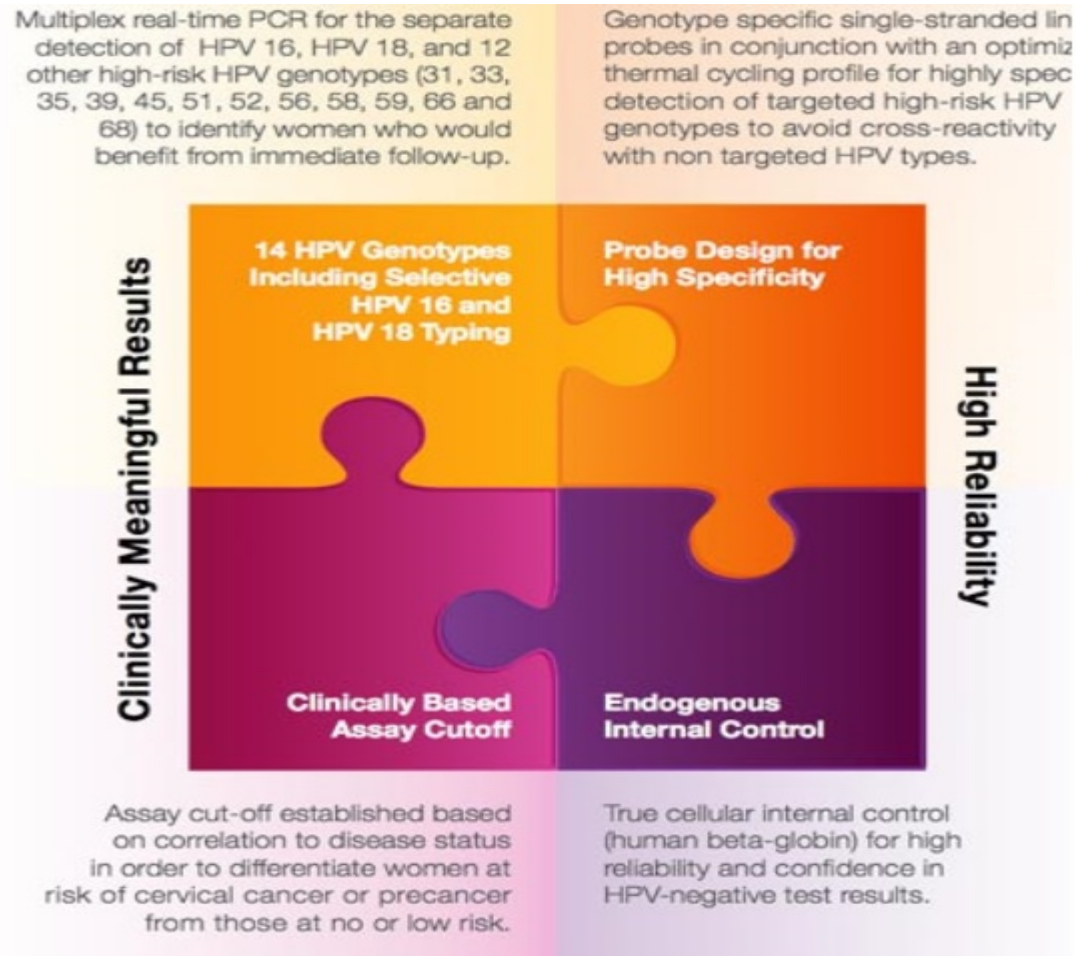
JNCI

# 14 High-risk HPV with 16/18 Genotyping Real-time PCR Kit

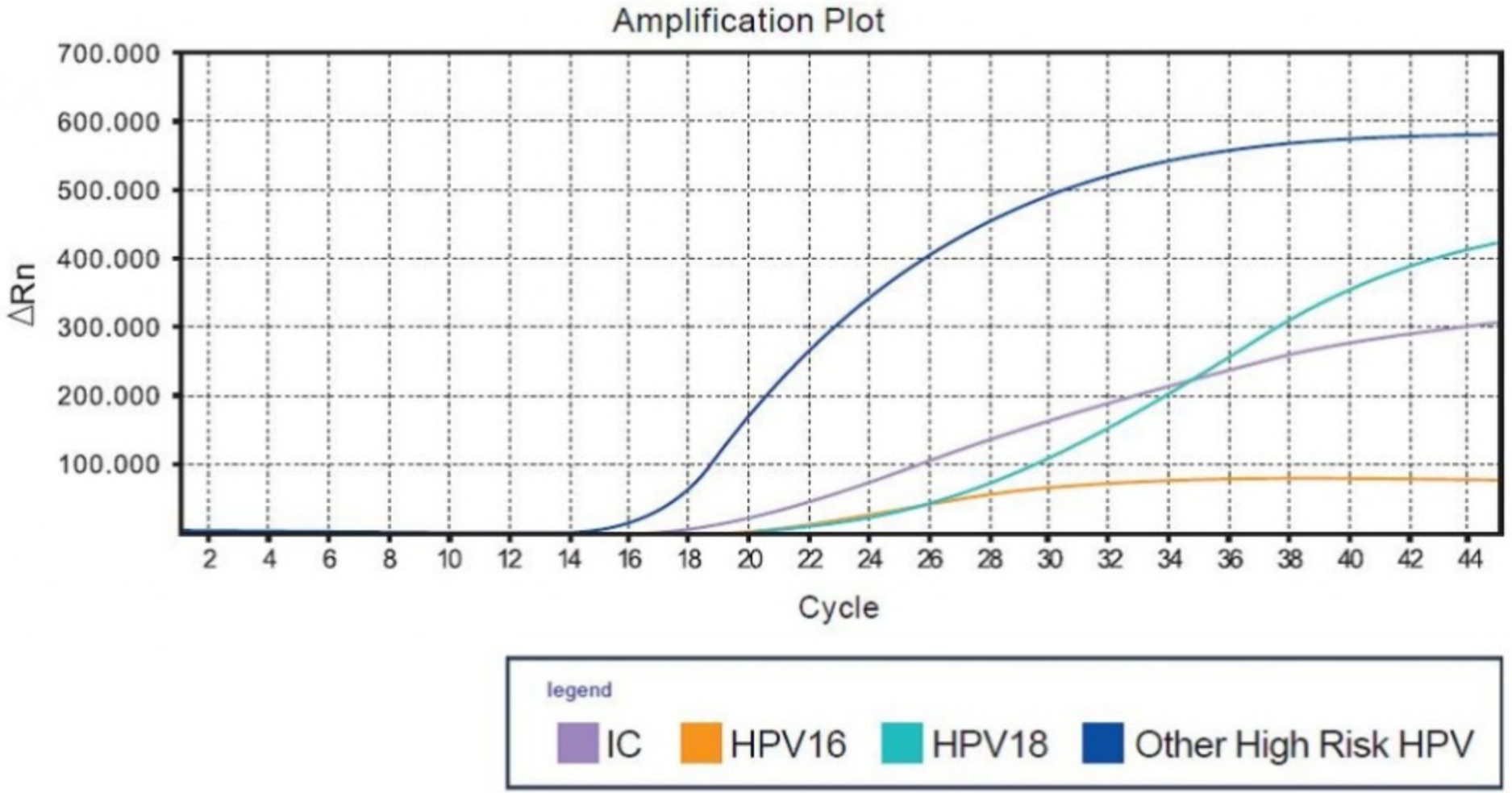
- 14 high-risk HPV types: HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68
- Specific Detection of 2 high-risk HPV genotypes: HPV 16 & HPV 18
- Cellular IC for the monitoring of the entire process


- 12 HR: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68
- Γονοτύπιση: 16, 18

# Abbott Real Time High Risk (HR) HPV




- 12 HR: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68
- Γονοτύπιση: 16, 18


Amplification Plot
700.000
600.000
500.000
400.000
300.000
200.000
100.000
ΔRn
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
42
44
Cycle
legend
IC
HPV16
HPV18
Other High Risk HPV

# Μέθοδοι που δε βασίζονται στο PCR

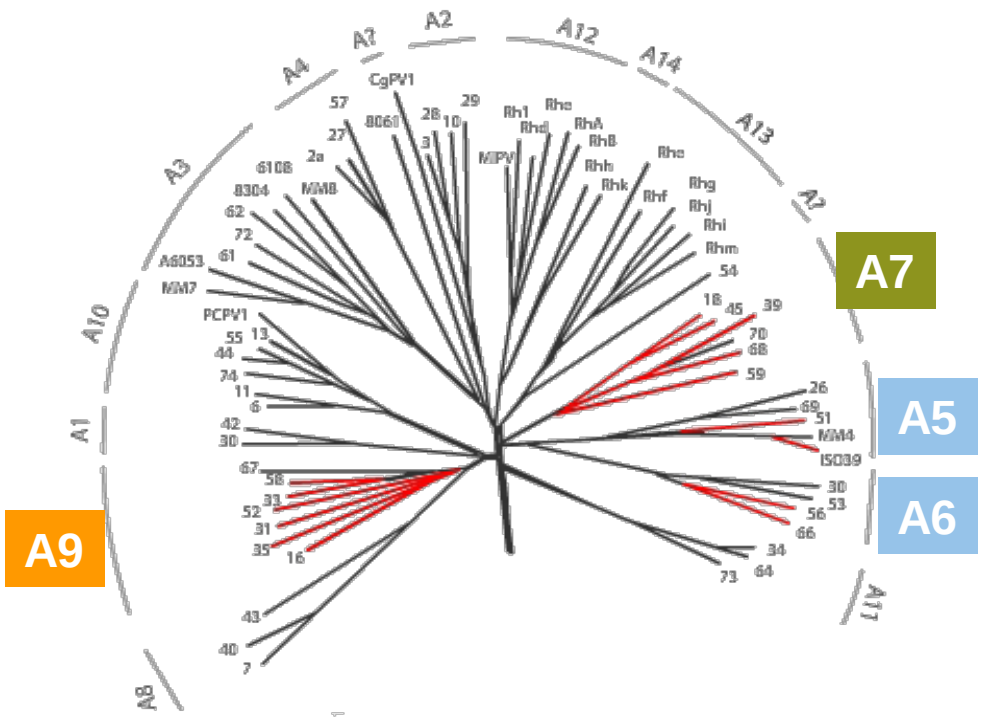
- ***digene* Hybrid Capture 2**
- **Cervista®**

# *digene* Hybrid Capture 2

- Διαδικασία ανίχνευσης DNA:RNA υβριδίων με ενίσχυση σήματος και με χρήση χημειοφωταύγειας για την ποιοτική ανίχνευση 18 τύπων χαμηλού και υψηλού κινδύνου HPV
- Διαφοροποίηση σε δύο ομάδες HPV DNA
- Χαμηλού κινδύνου (6, 11, 42, 43, 44)
- Υψηλού κινδύνου (16, 18, 31, 33, 35, 45, 51, 52, 56, 58, 59, 68)
- Δεν μπορεί να προσδιοριστεί ο συγκεκριμένος τύπος που υφίσταται

# Cervista™ HPV HR Test Design

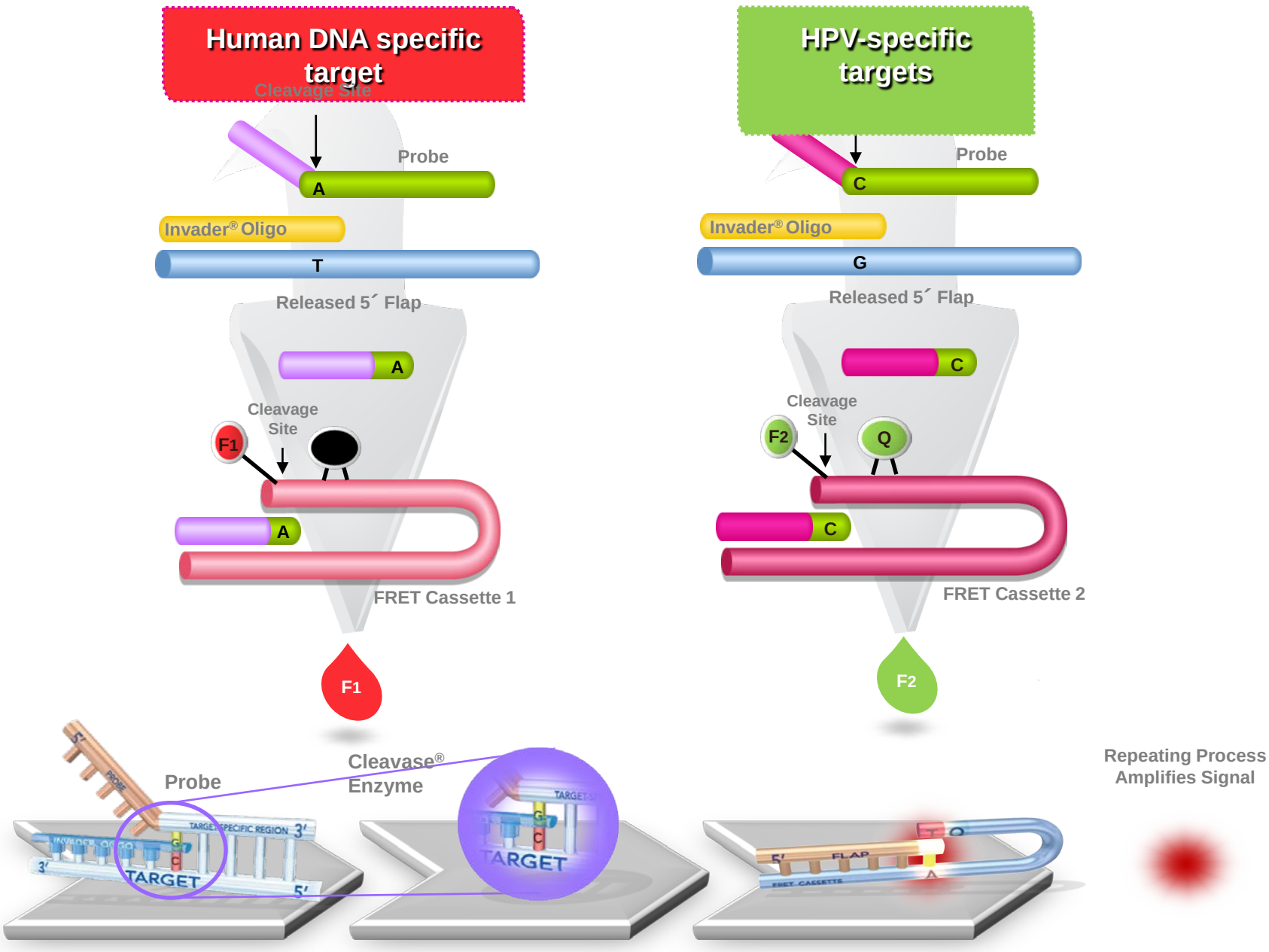
- Developed from phylogenetic tree of HPV strains A-superfamily, based on the complete L1 region
- Cervista HPV is specific for high-risk types selected from the A5/A6, A7 and A9 virus species:

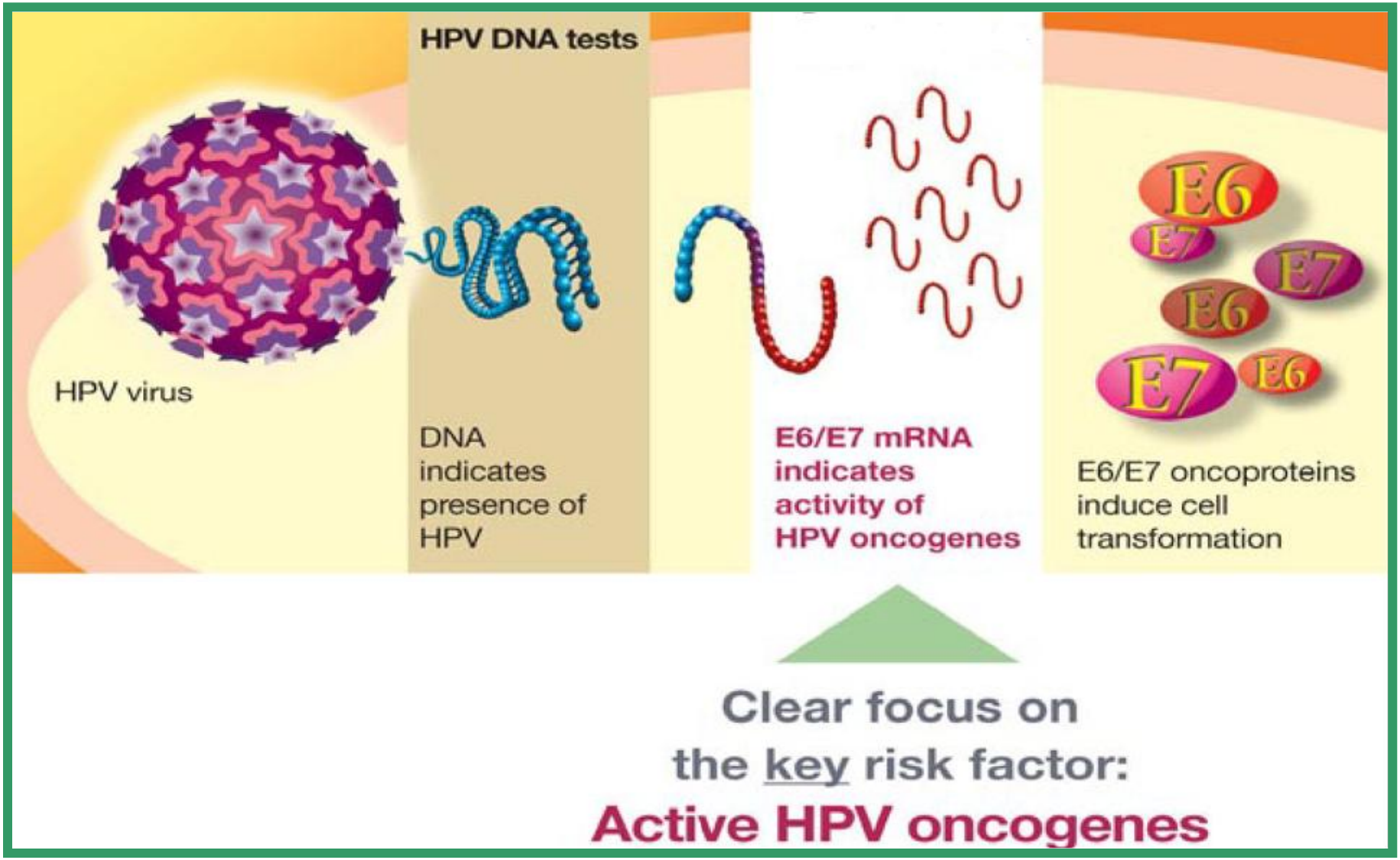



| | |
|---|---|
| A5/A6 | 51, 56, 66 |
| A7 | 18, 39, 45, 59, 68 |
| A9 | 16, 31, 33, 35, 52, 58 |

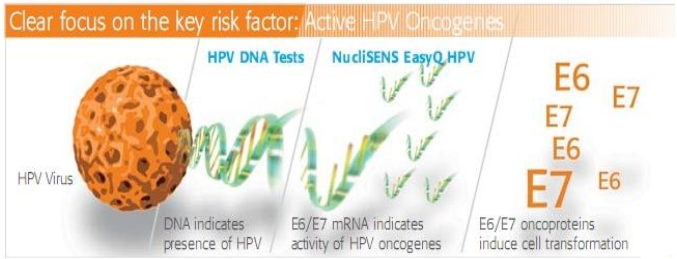
de Villiers et al., Virology, 2004

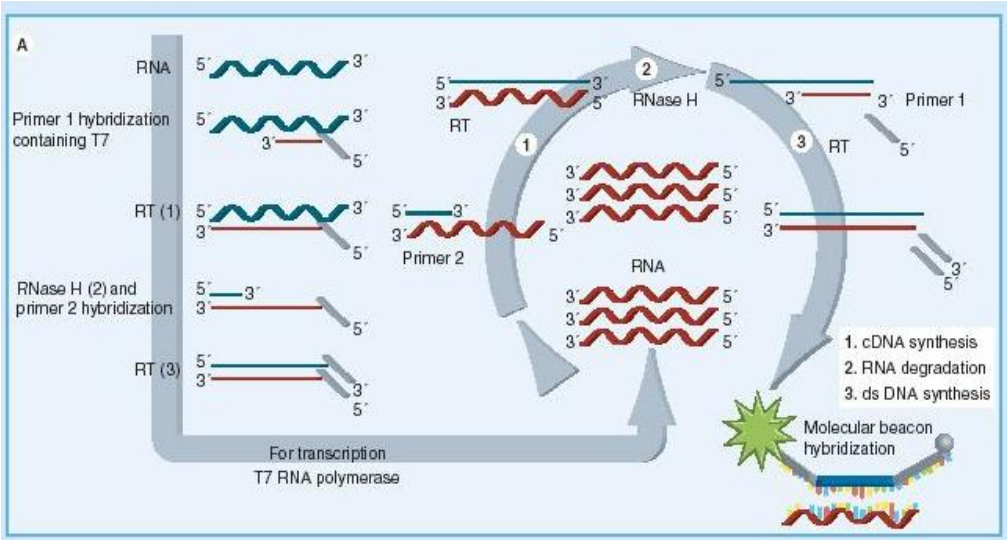
# Invader® Chemistry

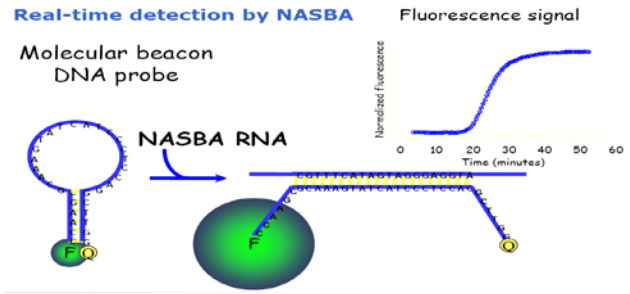


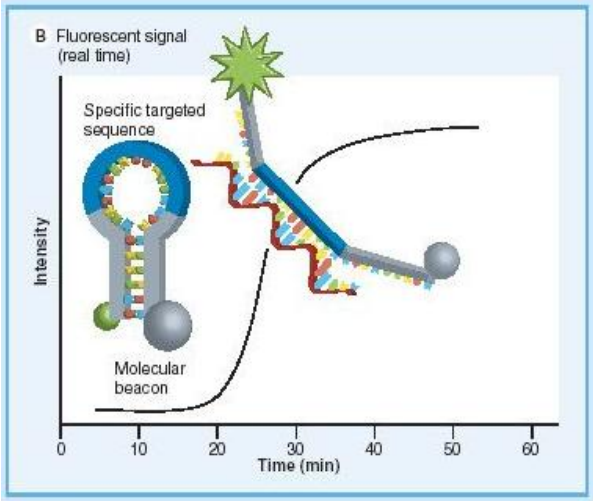

HPV DNA tests
HPV virus
DNA indicates presence of HPV
E6/E7 mRNA indicates activity of HPV oncogenes
E6
E7
E7
E6
E7
E6
E6/E7 oncoproteins induce cell transformation
Clear focus on the key risk factor:
Active HPV oncogenes

# NucliSens EasyQ® HPV (Biomerieux, France)



# Flow cytometric determination of E6/E7 mRNA of high risk HPV types

- Flow cytometer -488 nm argon laser

**Oncotect® protocol**

**1ml of ThinPrep (max $10^6$ cells)**

↓

**Fixation, permeabilization**
(IncellFP incellDx® )
**(90 min)**

↓

**Hybridization with probe coctail (30 min)**

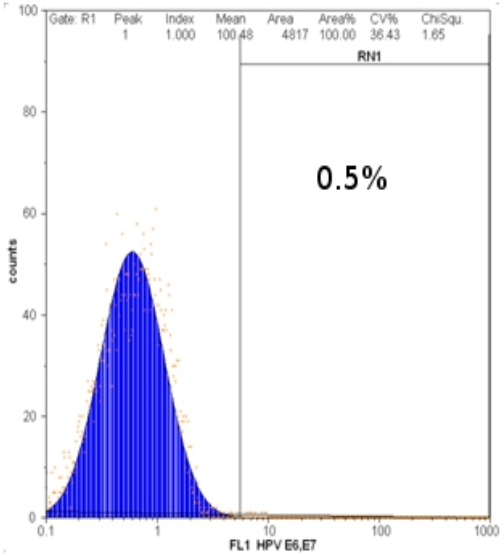
↓

**Strigent wash (15 min)**

→ **Count on flow cytometer (5 minutes)**

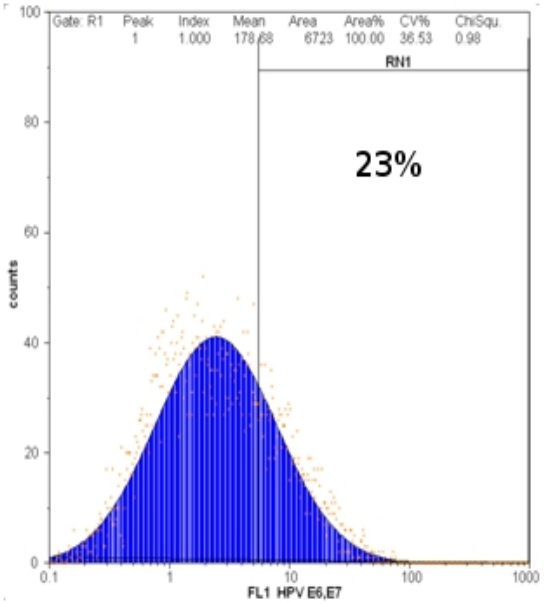

- **Combines in-situ hybridization with FC**
- **Screen cervical samples for HPV E6/E7 mRNA**
- **Non-time consuming and non labor-intensive workflow**
- **Quantifies transcripts in a cell-by-cell basis**

A




A “within normal limits” sample. HPV E6, E7 mRNA expression is quantified on a logarithmic scale on the x-axis and the percentage of E6, E7 mRNA overexpressing cells is also quantified.

B




A CIN 2 diagnosed sample demonstrating E6, E7 mRNA overexpression in 23% of ectocervical cells.

# Συμπεράσματα

•Πληθώρα δημοσιεύσεων επιχειρούν να διαλευκάνουν το ρόλο κάθε τεστ ανίχνευσης του ιού

•Συγκλίνουν στο συμπέρασμα ότι δεν υπάρχει ένα τεστ που από μόνο του μπορεί να υπολογίσει τον κίνδυνο ανάπτυξης σοβαρών αλλοιώσεων

•Ο συνδυασμός του τεστ-Παπανικολάου με μία ή περισσότερες μεθόδους ανίχνευσης του ιού μπορούν να αυξήσουν την ειδικότητα και την ευαισθησία στην πρόβλεψη της νόσου

**Προσωποποιημένη και ακριβής λήψη απόφασης**

# Ενδεικτική Βιβλιογραφία


•Coquillard, G., Palao, B., & Patterson, B. K. (2011). Quantification of intracellular HPV E6/E7 mRNA expression increases the specificity and positive predictive value of cervical cancer screening compared to HPV DNA. *Gynecologic Oncology, 120*(89-93.

•Cuzick, J., Arbyn, M., Sankaranarayanan, R., Tsu, V., Ronco, G., Mayrand, M. H., Dillner, J., & Meijer, C. J. (2008). Overview of human papillomavirus-based and other novel options for cervical cancer screening in developed and developing countries. *Vaccine*

•Mayrand, M. H., Duarte-Franco, E., Rodrigues, I., Walter, S. D., Hanley, J., Ferenczy, A., Ratnam, S., Coutlee, F., & Franco, E. L. (2007). Human papillomavirus DNA versus Papanicolaou screening tests for cervical cancer. *New England Journal of Medicine, 357*(16)*,* 1579-1588.

•Molden, T., Kraus, I., Karlsen, F., Skomedal, H., & Hagmar, B. (2006). Human papillomavirus E6/E7 mRNA expression in women younger than 30 years of age. *Gynecologic Oncology, 100*(95-100.

•Spathis, A., Kottaridi, C., Chranioti, A., Meristoudis, C., Chrelias, C., Panayiotides, I. G., Paraskevaidis, E., & Karakitsos, P. (2012). mRNA and DNA detection of human papillomaviruses in women of all ages attending two colposcopy clinics. *PloS one, 7*(11)*,* e49205.

# Ευχαριστώ για την προσοχή σας!