HPV infektion – Cancerudvikling og diagnostik

belyst udfra virus molekylære egenskaber

Odense 7.3.2009

BN 2009

100 år med Virus og Cancer

- 1908- Ellerman & Bang- Leukemia i kyllinger (ALV)
- 1911- Rous- Sarcomer i kyllinger (RSV)
- 1972- Burkitts Lymphom & NPC (EBV)

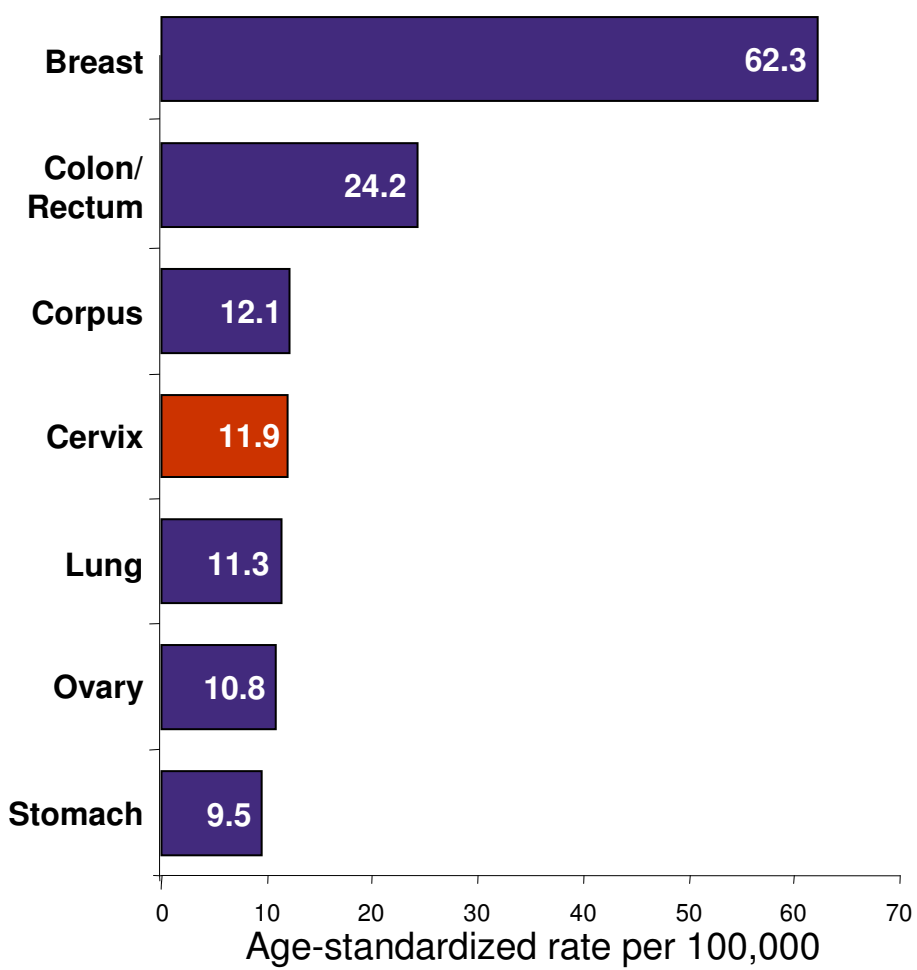
  (Henle, Klein, Epstein, zurHausen, Nonoyama, Pagano)
- **1962- Cervix Cancer (HSV)** (Rawls,Melnik,Naib,Nahmias, *Roizman*, *McDougall*)
- 1974- Hepatocellular Carcinoma (HBV, HCV)

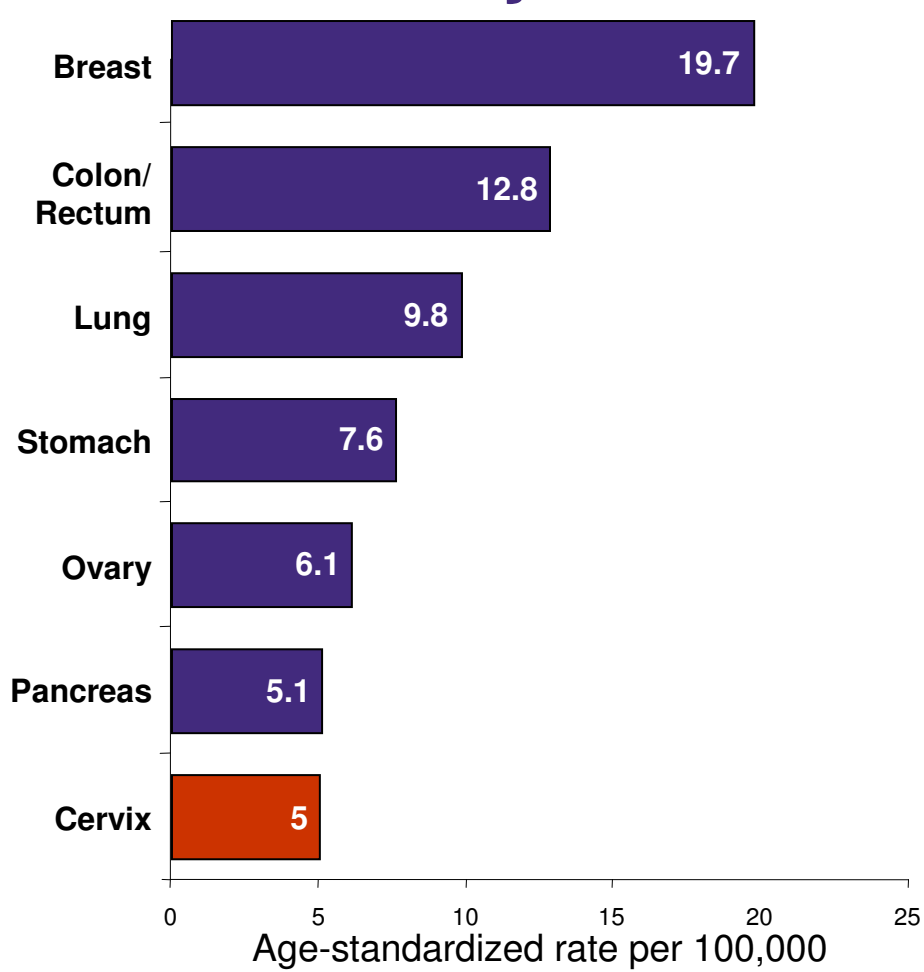
  (Reed, Liaw, Blumberg, Shikata, Williams, Bartok)
- **1975- Cervix Cancer (HPV)** (zurHausen, *Orth, Jablonska*)
- **2008- H zur Hausen Nobelpris vinder for HPV /** cancer opdagelsen

- HPV og Cancer udvikling
- HPV vaccine
- Hvilke vigtige spørgsmål står vi overfor?
- Hvilke HPV tests skal vi vælge

Hyppigste cancers i kvinder: incidence og mortalitet

Europe

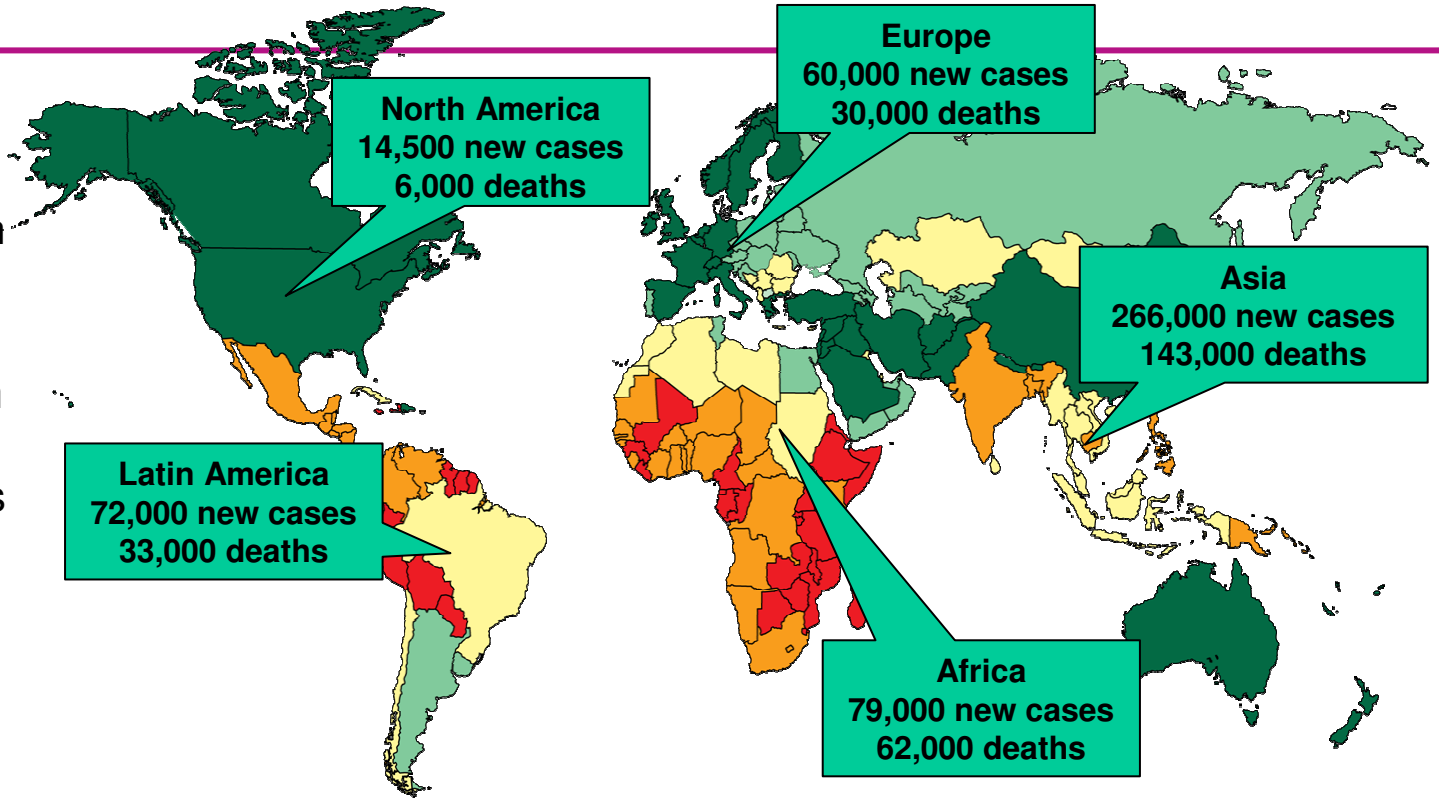




Ferlay J, *et al. GLOBOCAN 2002 Cancer Incidence, Mortality and Prevalence Worldwide.* IARC CancerBase; Lyon; 2004.

Global mortalitet per år

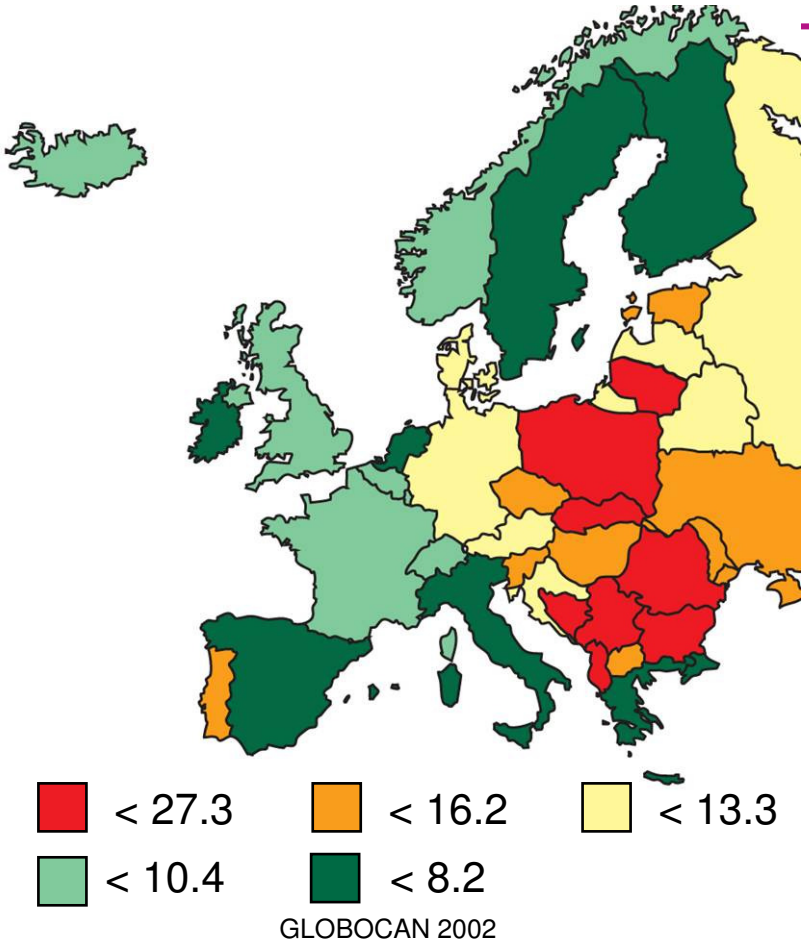
- Worldwide, every 2 minutes a woman dies of cervical cancer[1]
- The highest burden of disease (up to 80%) occurs in less developed regions[1] where there is a lack of effective screening programmes
- This demonstrates a clear medical need for new cervical cancer interventions



Cervical cancer mortality rates worldwide
Cases per 100,000 women per year

1. Ferlay J, *et al. GLOBOCAN 2002 Cancer Incidence, Mortality and Prevalence Worldwide.* IARC CancerBase; Lyon, 2004.

Europæisk incidence

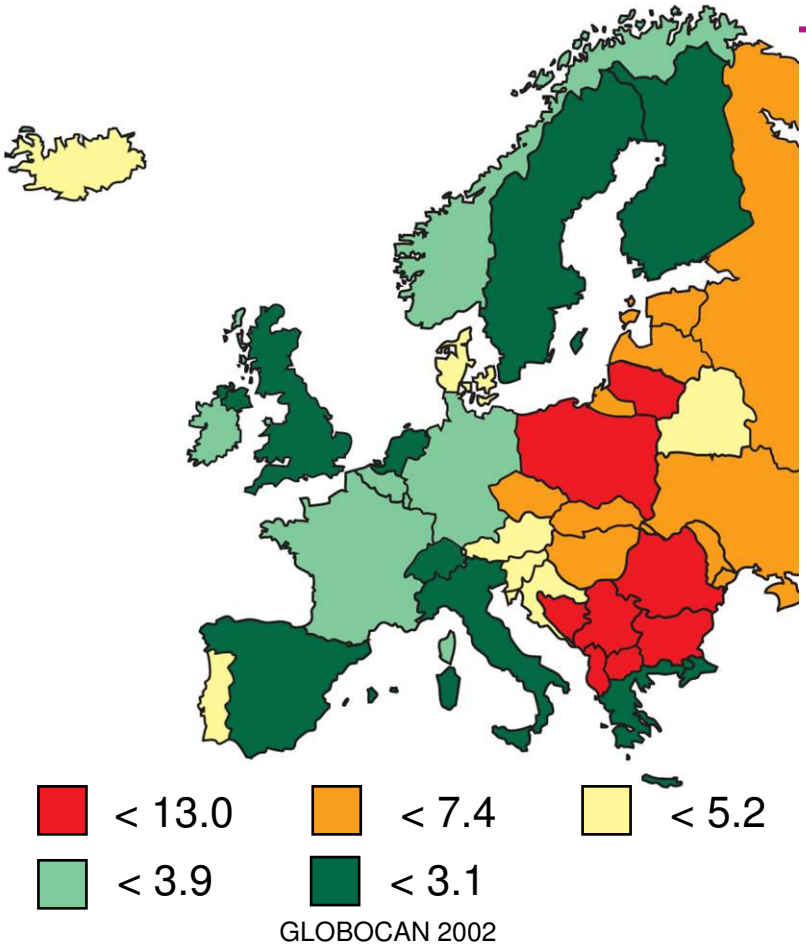


Cervical cancer incidence rates in Europe
Cases per 100,000 women per year

- Every year, across Europe:[1]
  - **60,000** women are diagnosed with cervical cancer
  - **30,000** women will die from the disease
- Age-standardized incidence rates are:[1]
  - Eastern Europe (14.5 per 100,000)
  - Northern Europe (9.0 per 100,000)
  - Southern Europe (10.7 per 100,000)
  - Western Europe (10.0 per 100,000)

1. Ferlay J, *et al. GLOBOCAN 2002 Cancer Incidence, Mortality and Prevalence Worldwide.* IARC CancerBase; Lyon, 2004.

Europæisk mortalitet

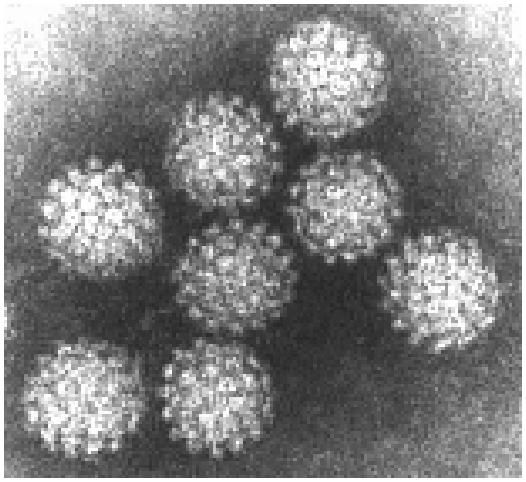


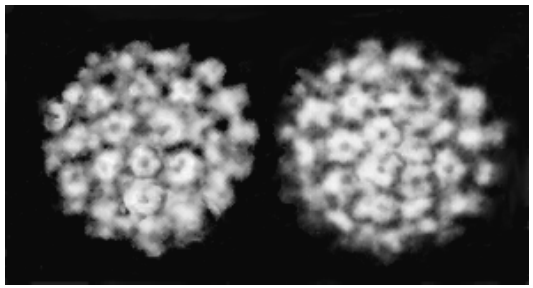
Cervical cancer mortality rates in Europe
Cases per 100,000 women per year

- Høj mortalitet rates in:[1]
  - Serbia and Montenegro (10.1 per 100,000)
  - Romania (13.0 per 100,000)
  - Slovakia (6.1 per 100,000)
- Lav mortalitet rates in:[1]
  - Finland (2.3 per 100,000)
  - The Netherlands (2.3 per 100,000)

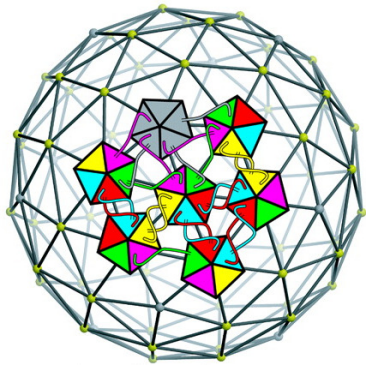
1. Ferlay J, *et al. GLOBOCAN 2002 Cancer Incidence, Mortality and Prevalence Worldwide.* IARC CancerBase; Lyon, 2004;
2. NHS cervical screening programme statistics. Accessed at http://www.cancerscreening.nhs.uk/cervical/statistics.html.

HPV infectiøs partikel





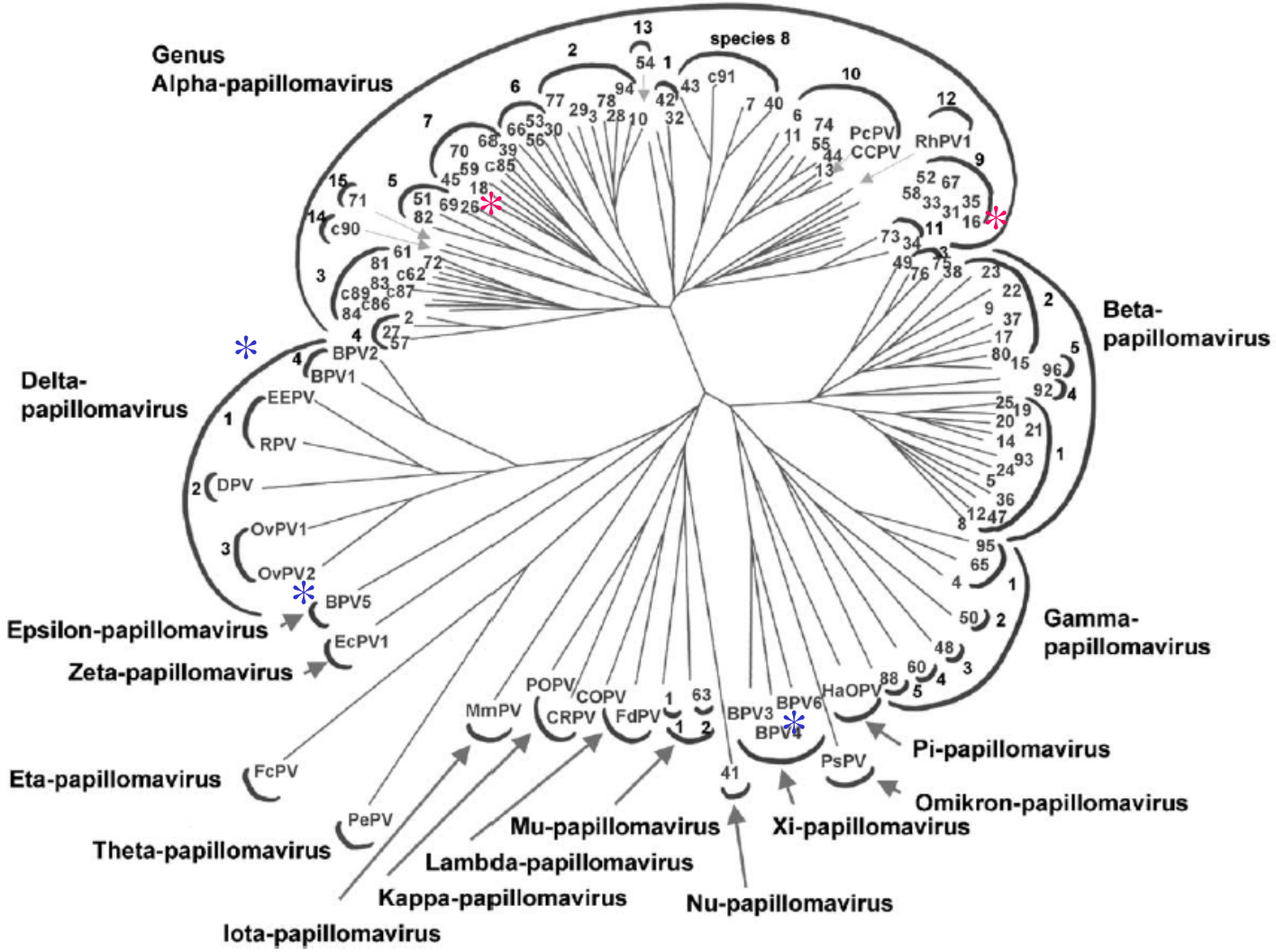
- Indeholder HPV genom
- Diameter: 600 Å
- 72 L1 pentamers og app. 12 L2 monomers



Modis *et al.* 2002 **EMBO 21**: 4754-4762

Classifikation af Papillomaviruses

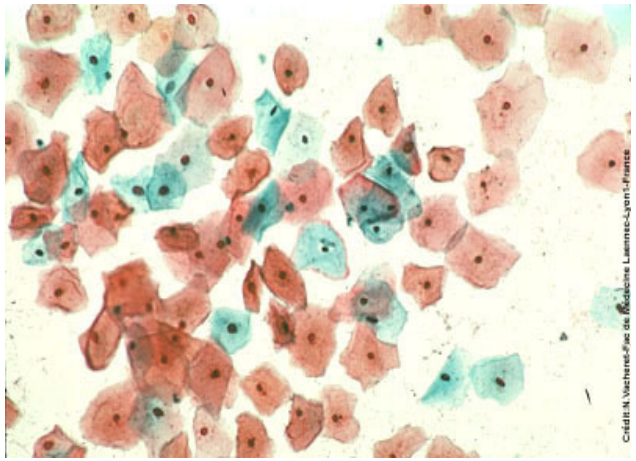
E.-M.de Villiers et al J.Virol 2004



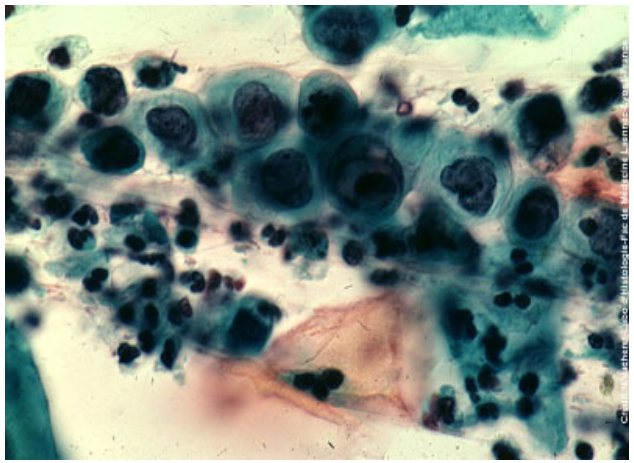
Cervix Cancer

- **Species 9:** HPV-16, -31,-33, -35, -52, og -58 er oftest i squamous cancers i exocervix

- **Species 7:** HPV-18, -39, -45, og -68 er almindeligvis i glandular tumors (adenocarcinomas) i den endocervicale kanal.



Normal PAP

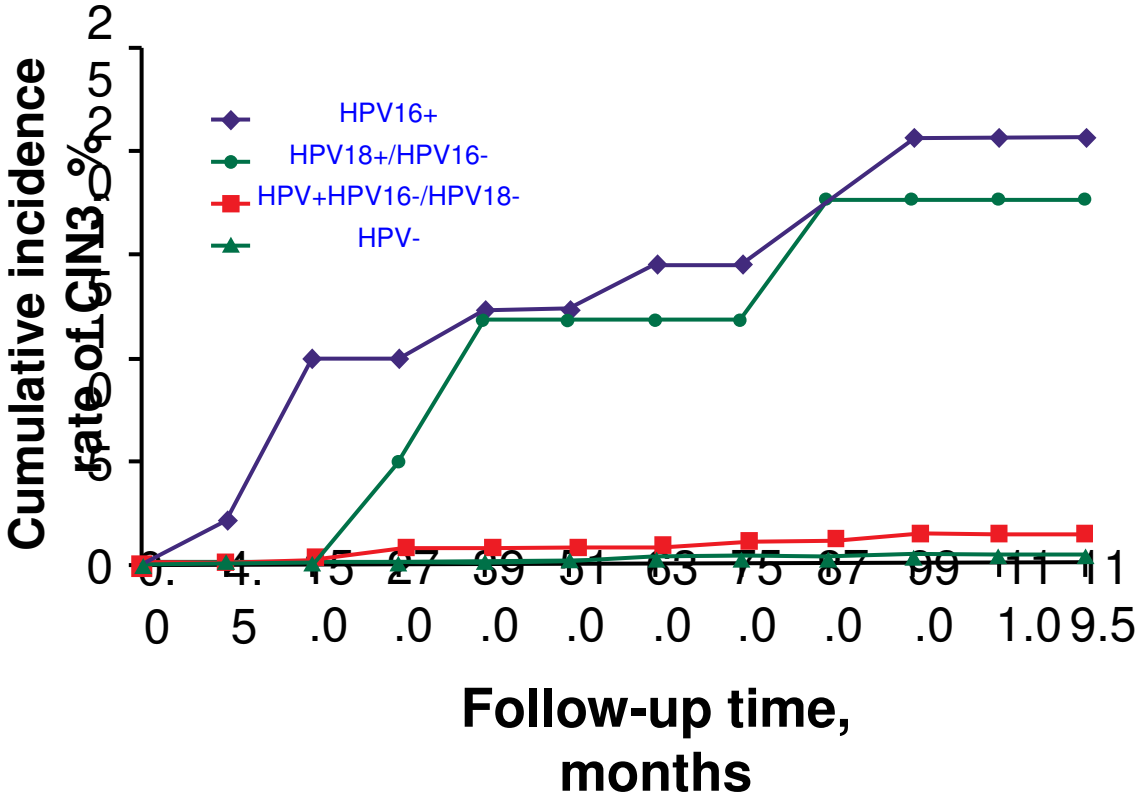


Carcinoma n situ

Cumulative risiko for CIN3+ associeret med HPV 16 og 18

- 13,000 kvinder over 30 år monitoreret over 10 år efter single HPV DNA test

- Incidence af CIN3+er **stærkt associated** med infection med HPV 16 eller HPV 18
- Risiko er **betydeligt** højere for kvinder positive for HPV 16 eller HPV 18
- Type-specifik HPV screening vil identicere kvinder med størst risiko



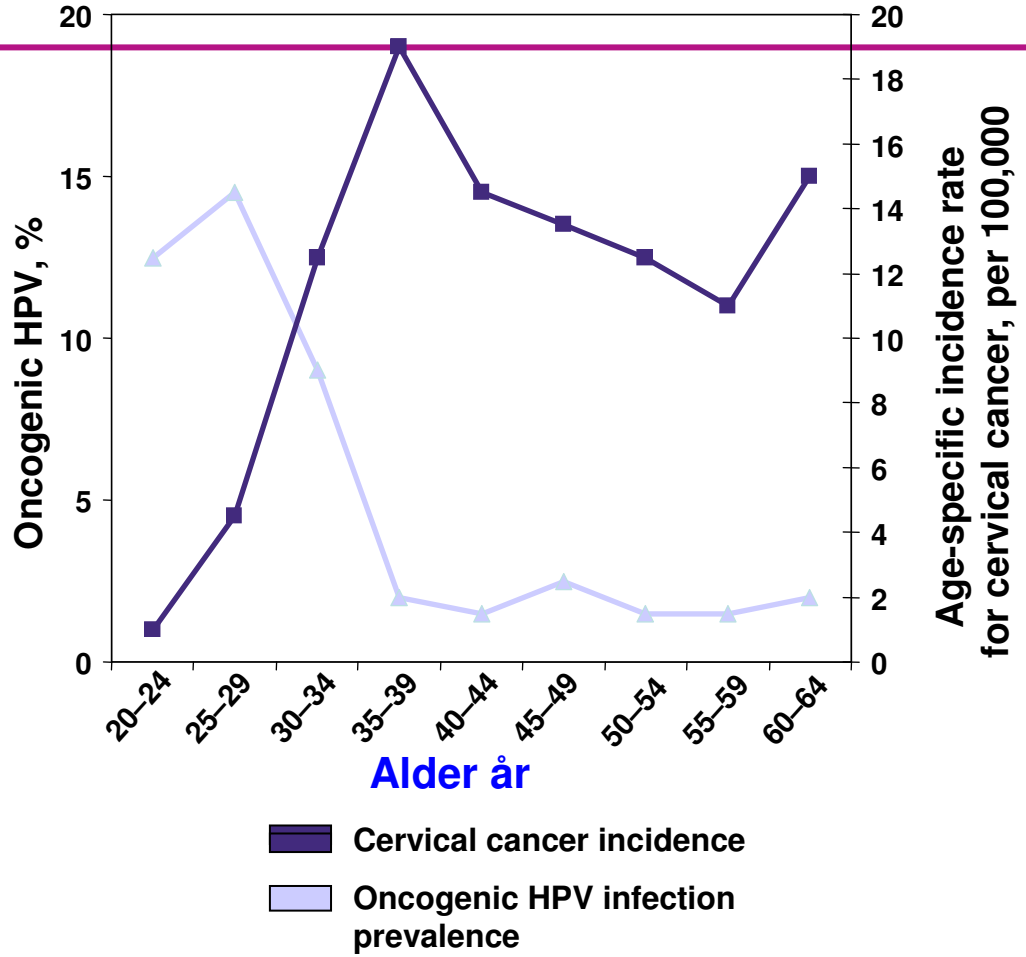
Khan MJ, *et al. J Natl Cancer Inst* 2005; **97**:1072–1079.

Alders specifik HPV incidence

- HPV infektion sker år før cervical cancer [1]
- Den højeste of cervical cancer frekvens ses hos kvinder over > 45 år[2]

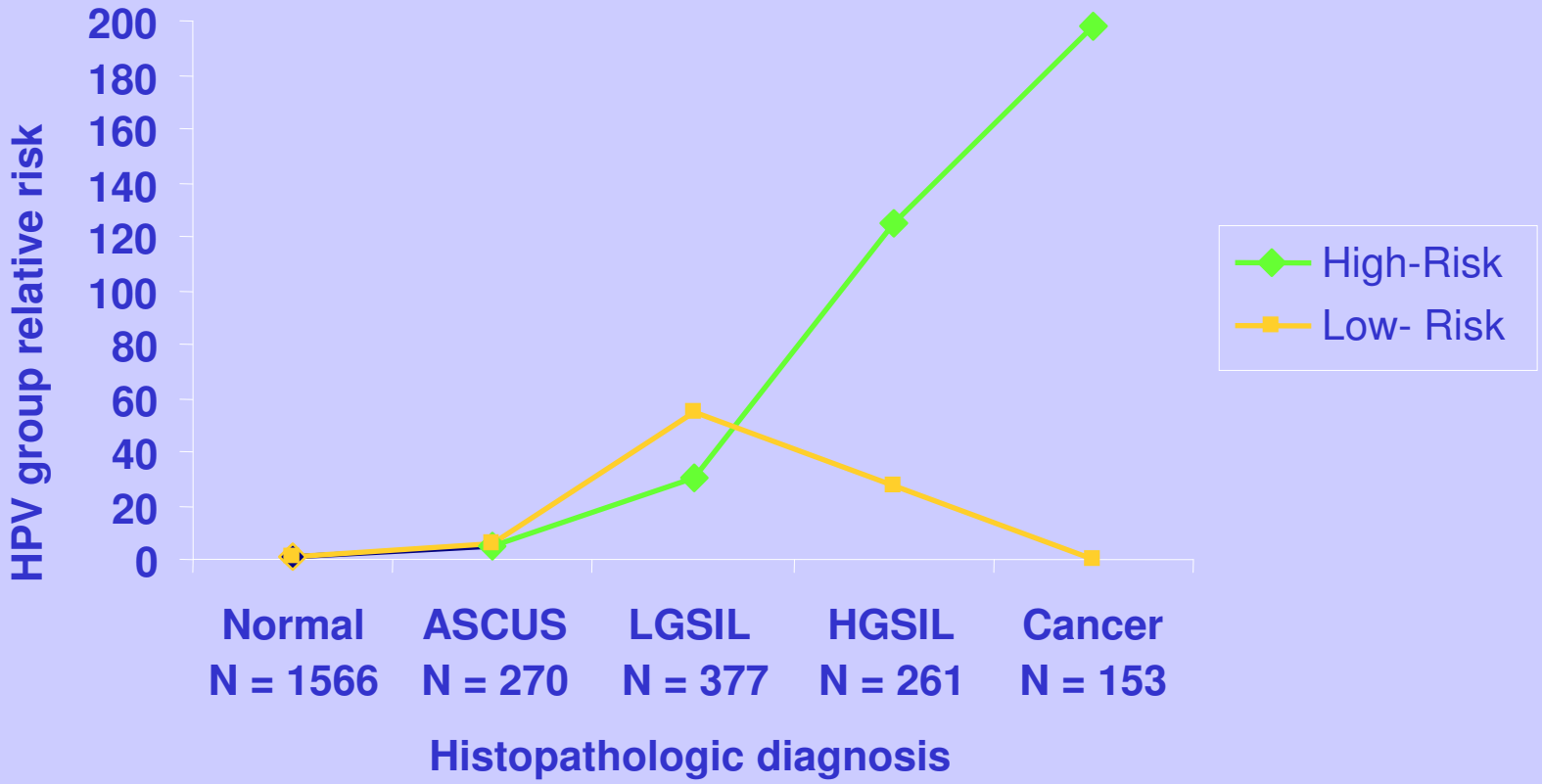
men

- 30% af cases ses i kvinder < 45 år[2]



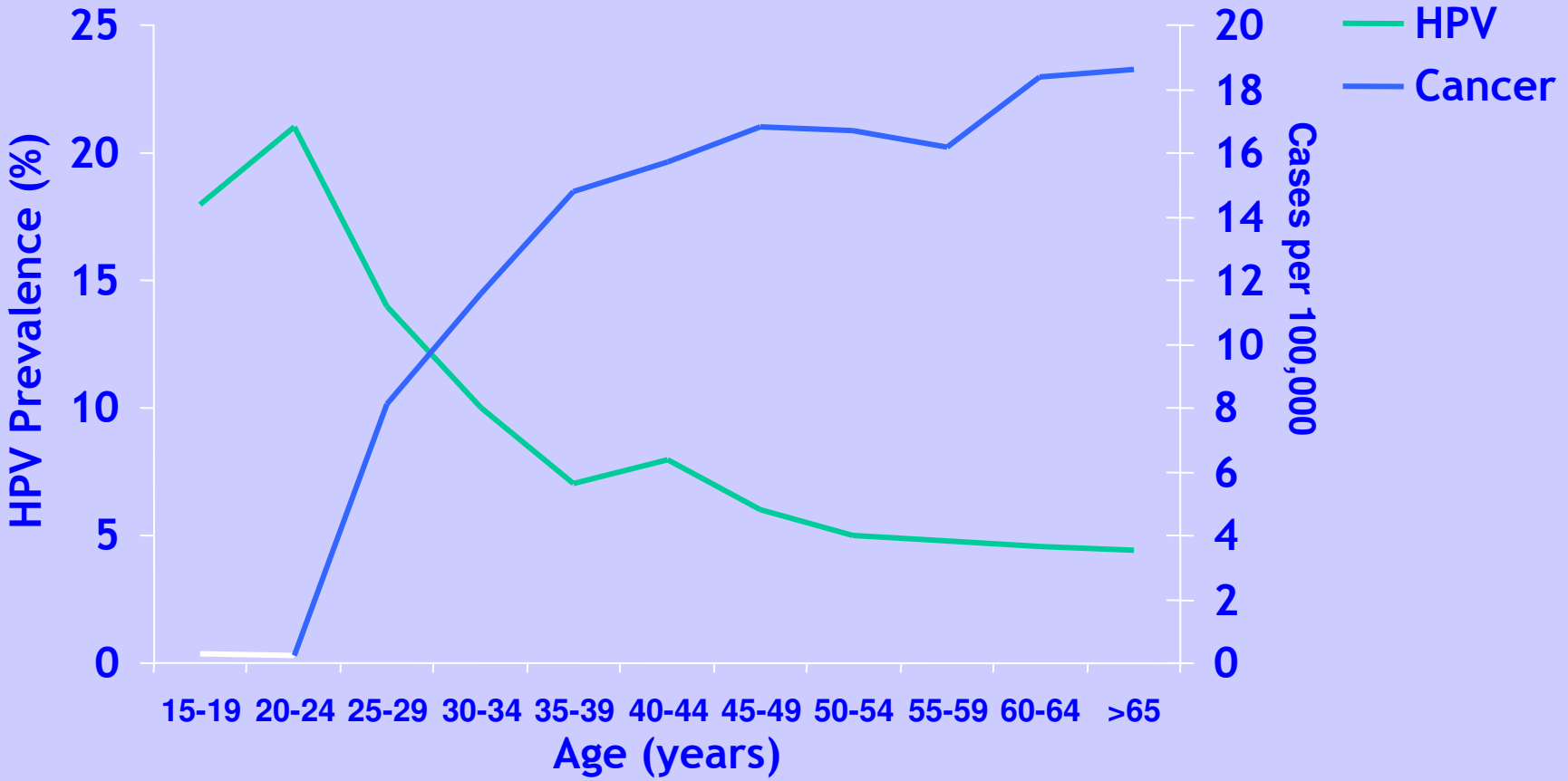
1. Bosch FX, *et al. J Clin Pathol* 2002; **55:**244–265.
2. Ferlay J, *et al. GLOBOCAN 2002 Cancer Incidence, Mortality and Prevalence Worldwide.* IARC CancerBase; Lyon, 2004.

Relativ Risiko for HPV Typer



Source: Lorincz et al., 1992. Obstet. Gynecol. 79:328-337.

HPV Prevalence og Cervix Cancer – alders afhængighed



Sources: NCI SEER Data, 1990-94; Melkert et al., 1993. Int J Canc 53:919.

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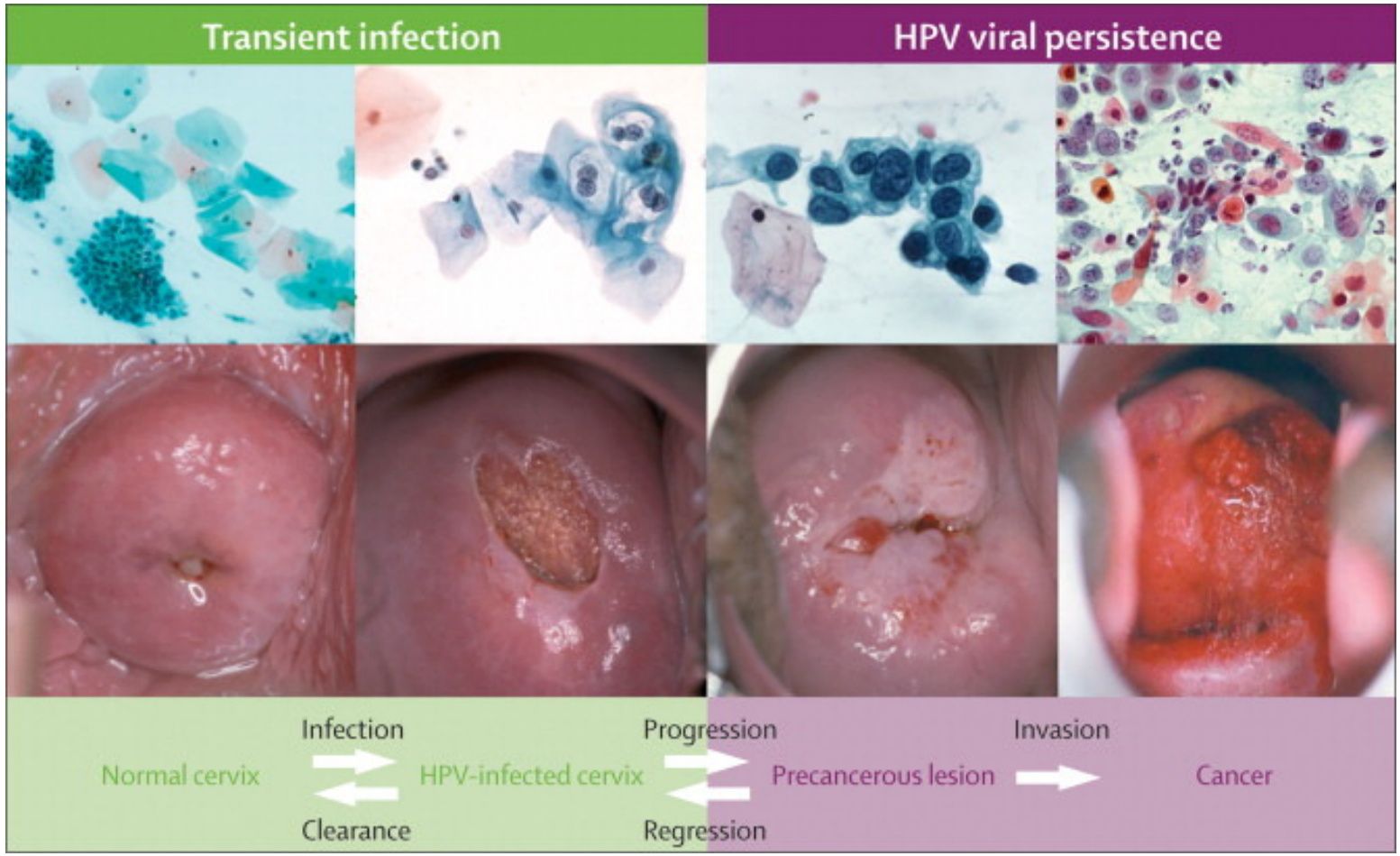
Clinisk progression

Ref. Schiffman et al Lancet 370,p890,2007



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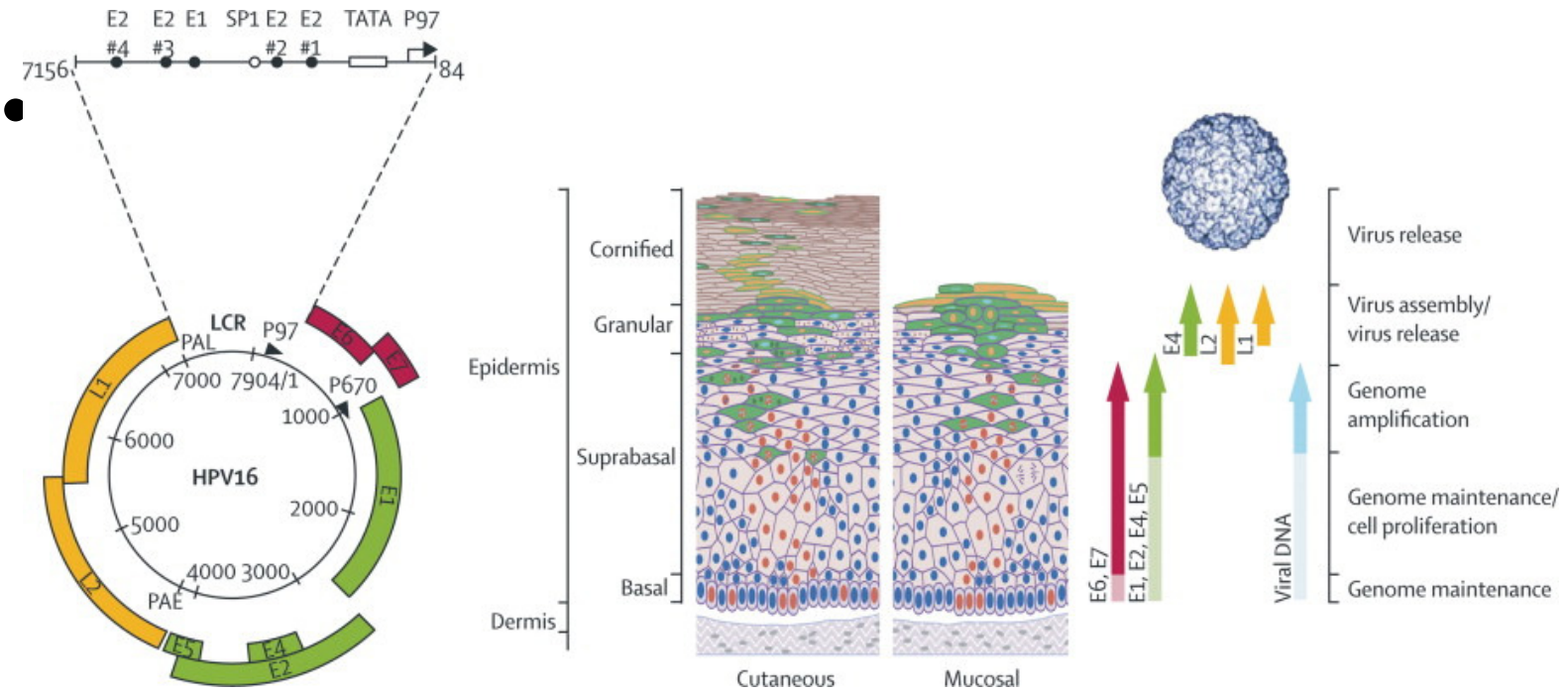
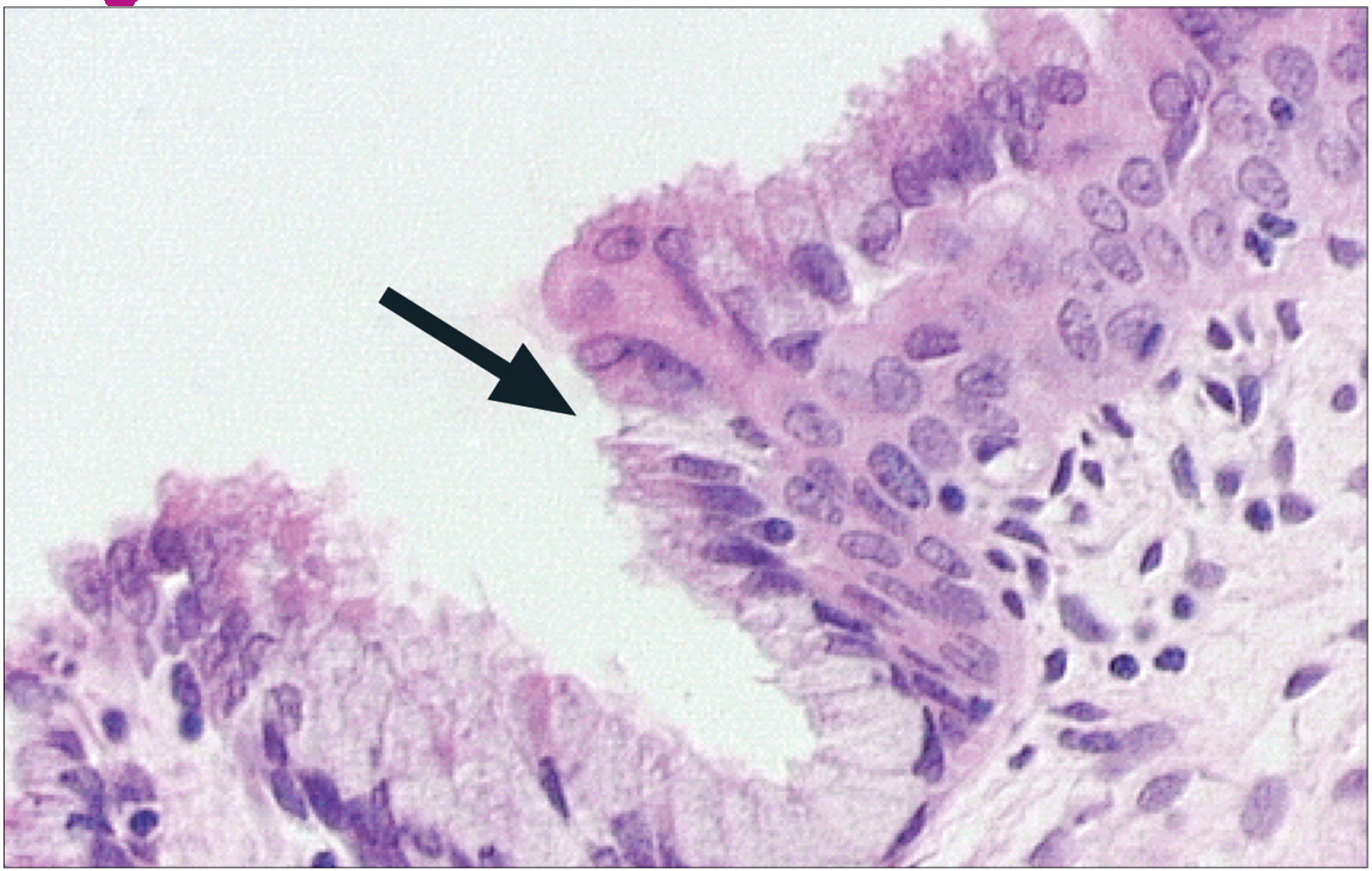
HPV genom og expression

Ref. Schiffman et al Lancet 370,p890,2007



Transformation zone-normal

Ref. Schiffman et al Lancet 370,p890,2007



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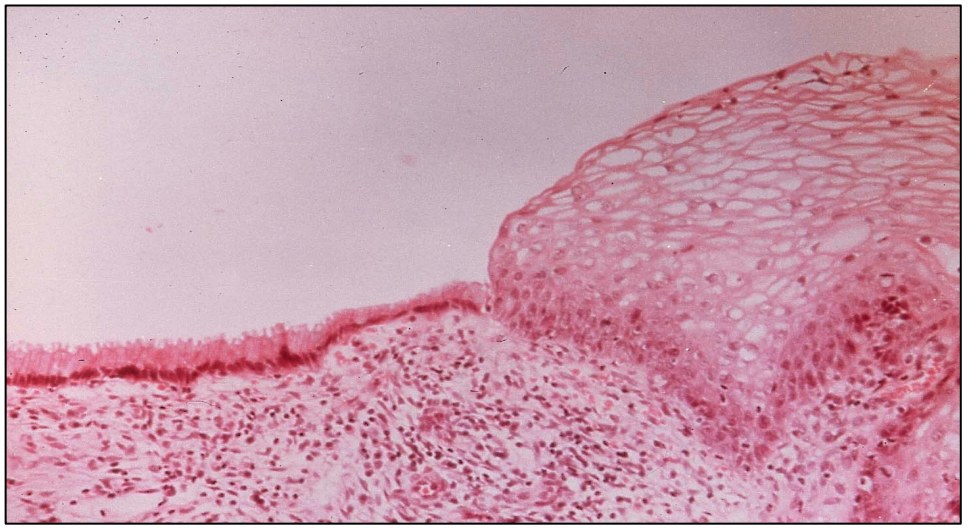
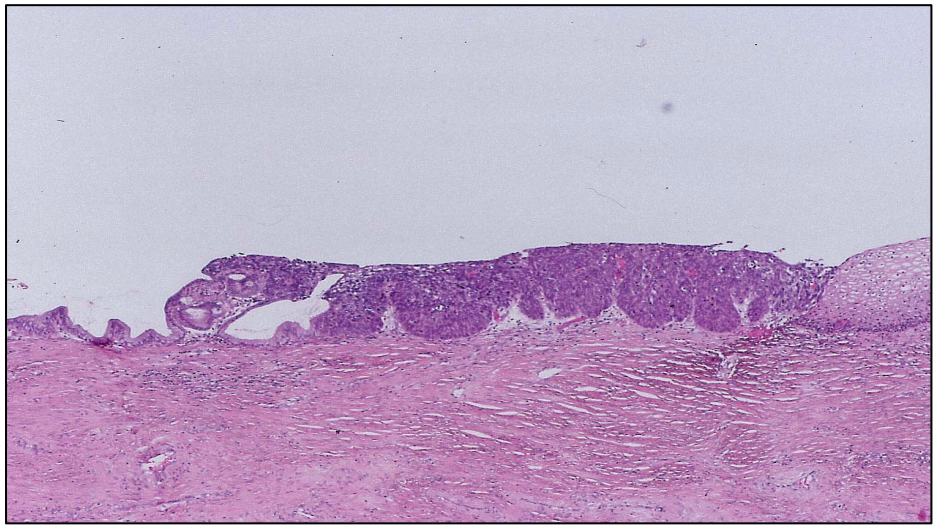
Cervical transformation zone er det primære target for carcinogenesis

Normal

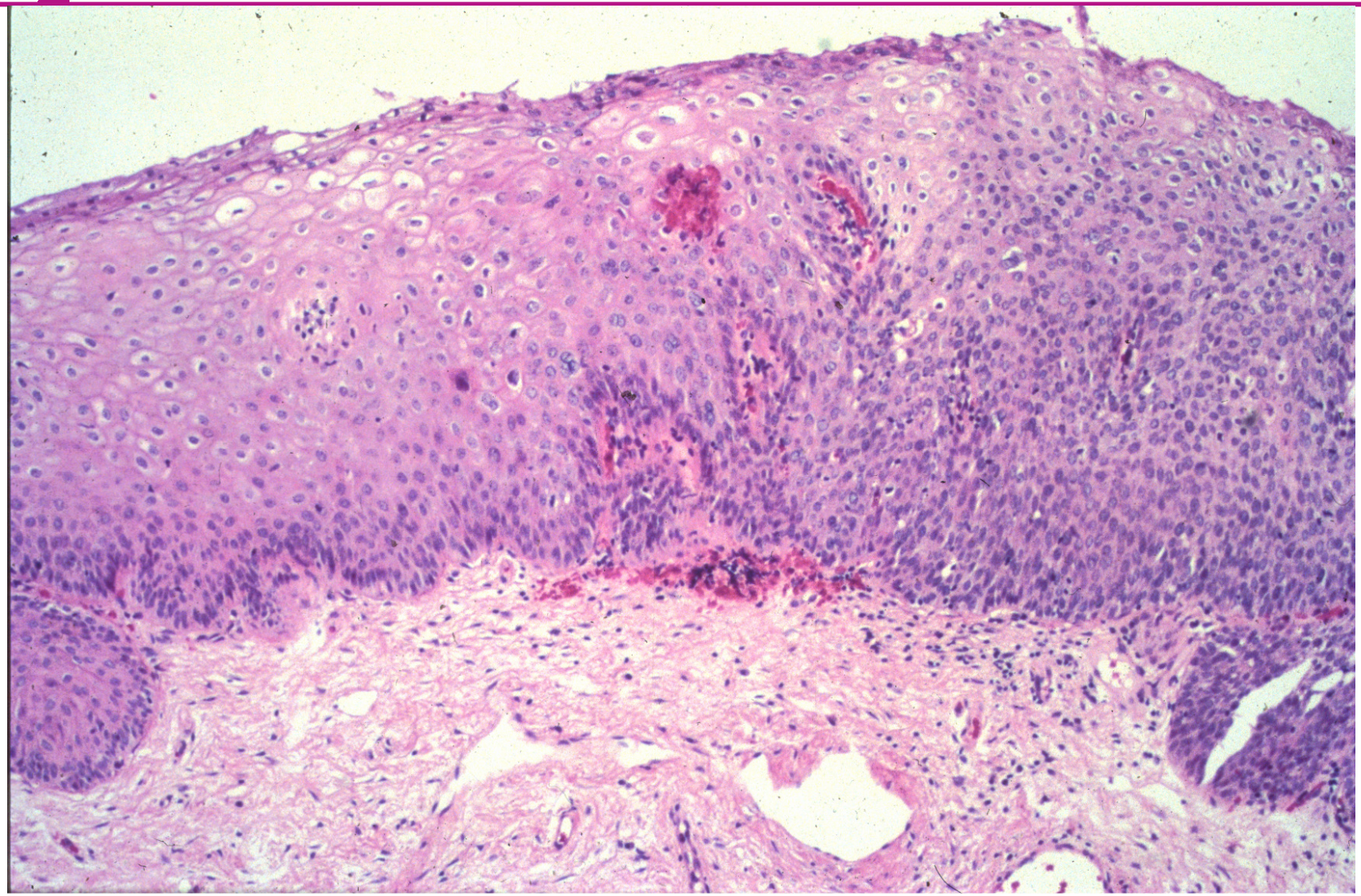


High-grade lesion

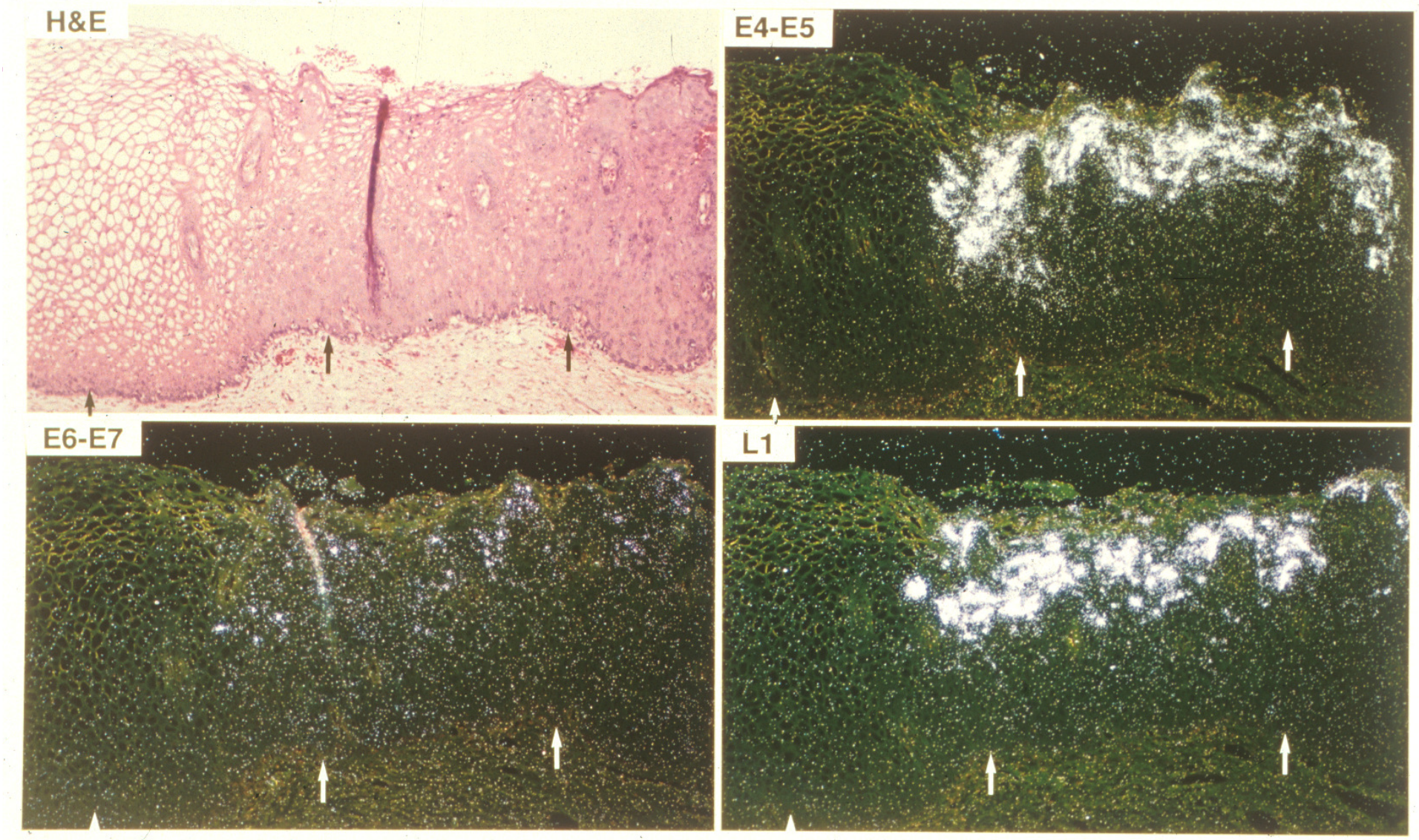


HPV-inficeret & transformeret

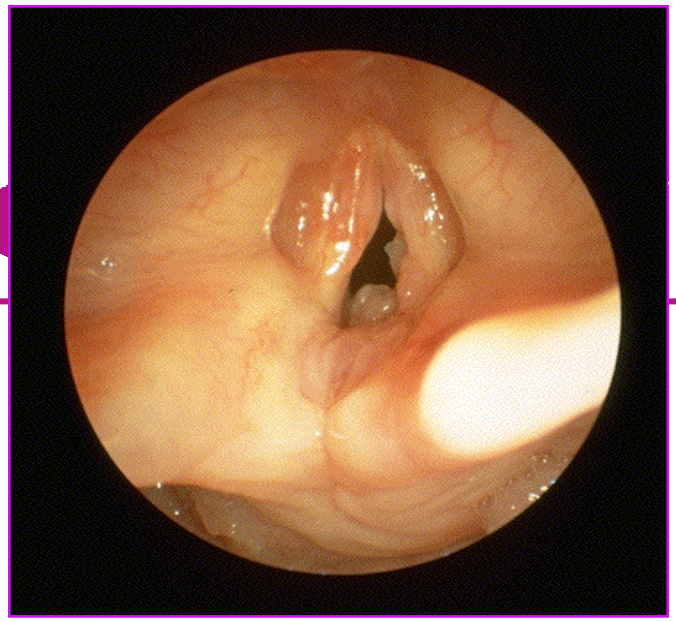
T.Broker 2007



HPV-16 mRNA Expression in Cervical HGSIL in an HIV-Positive Patient



STAGING



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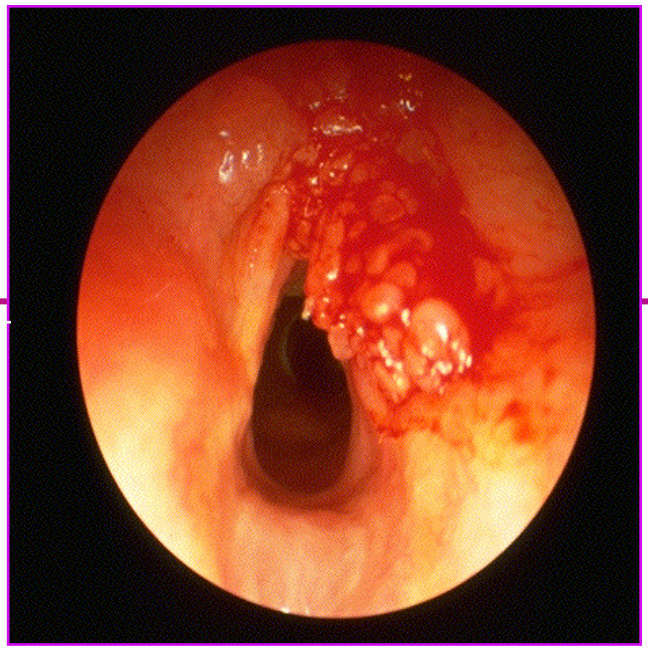

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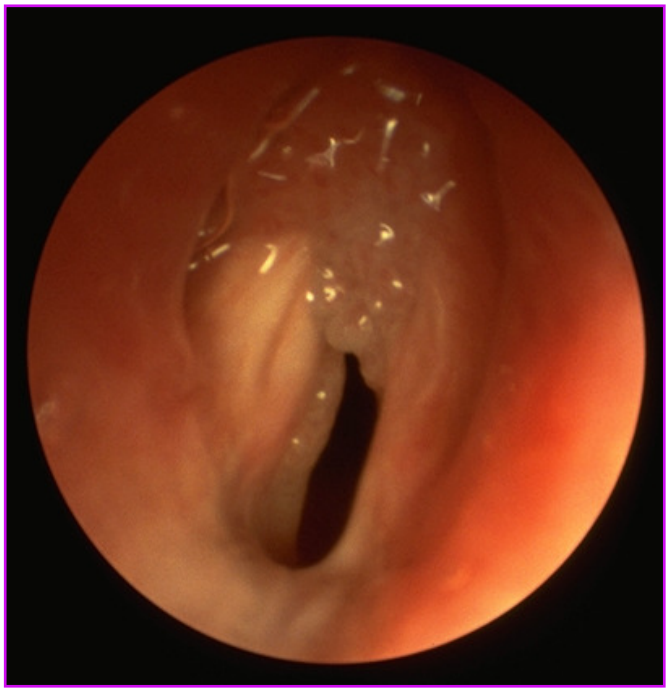

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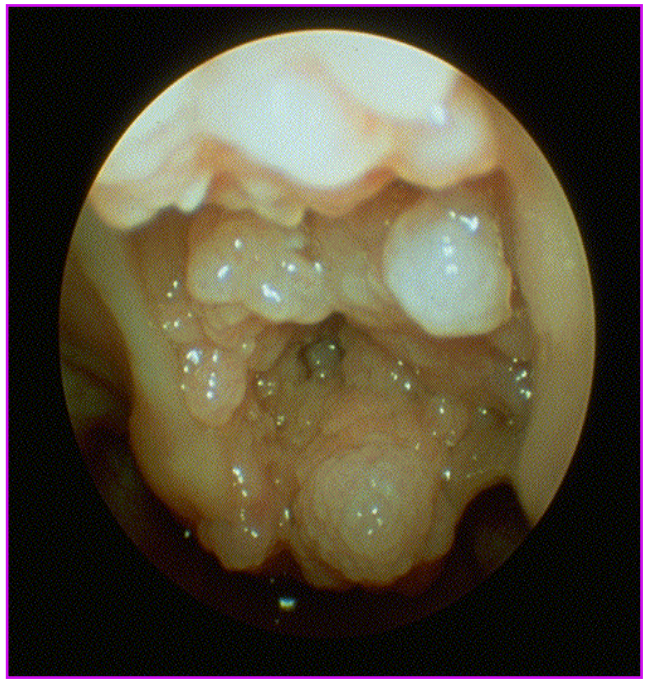

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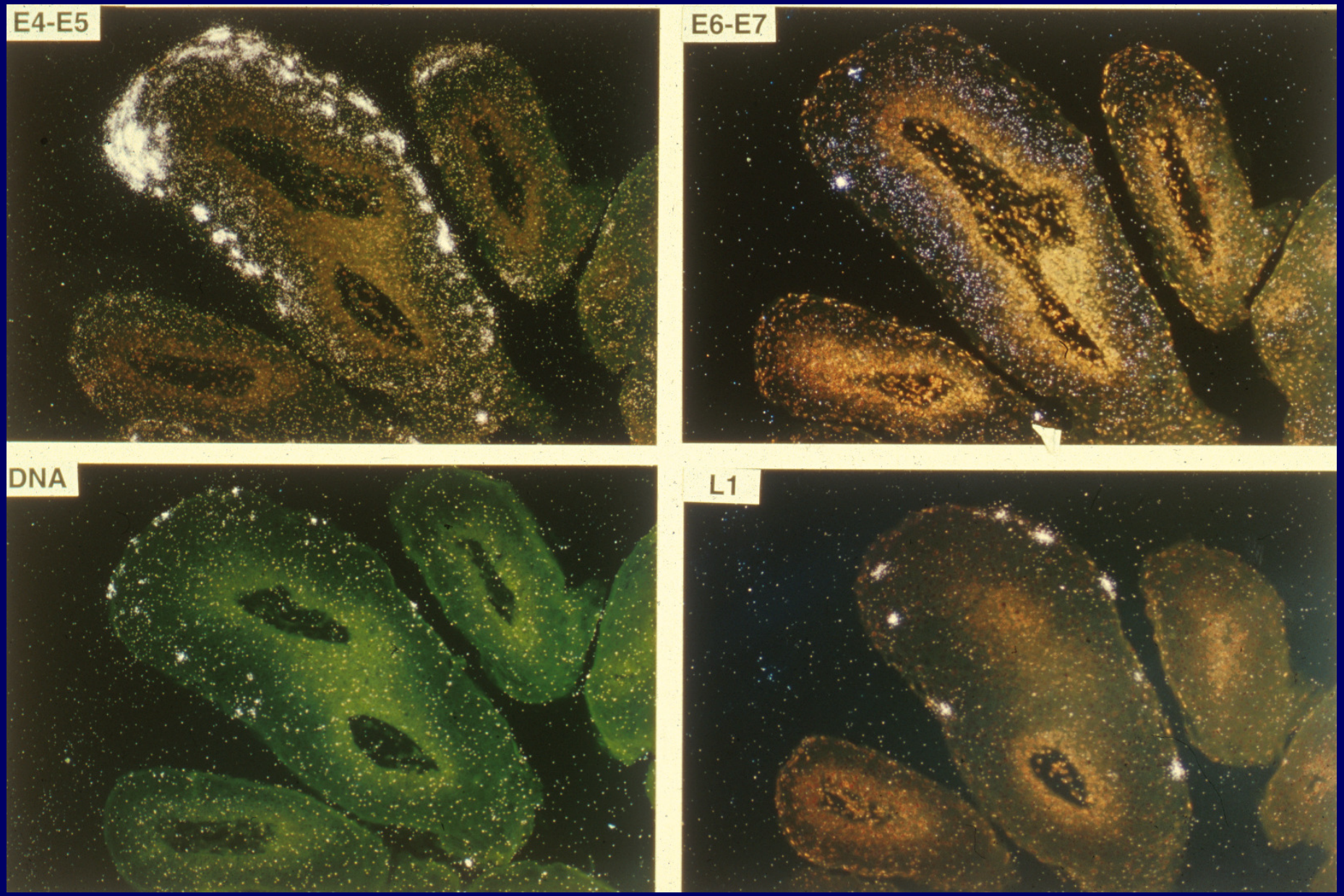
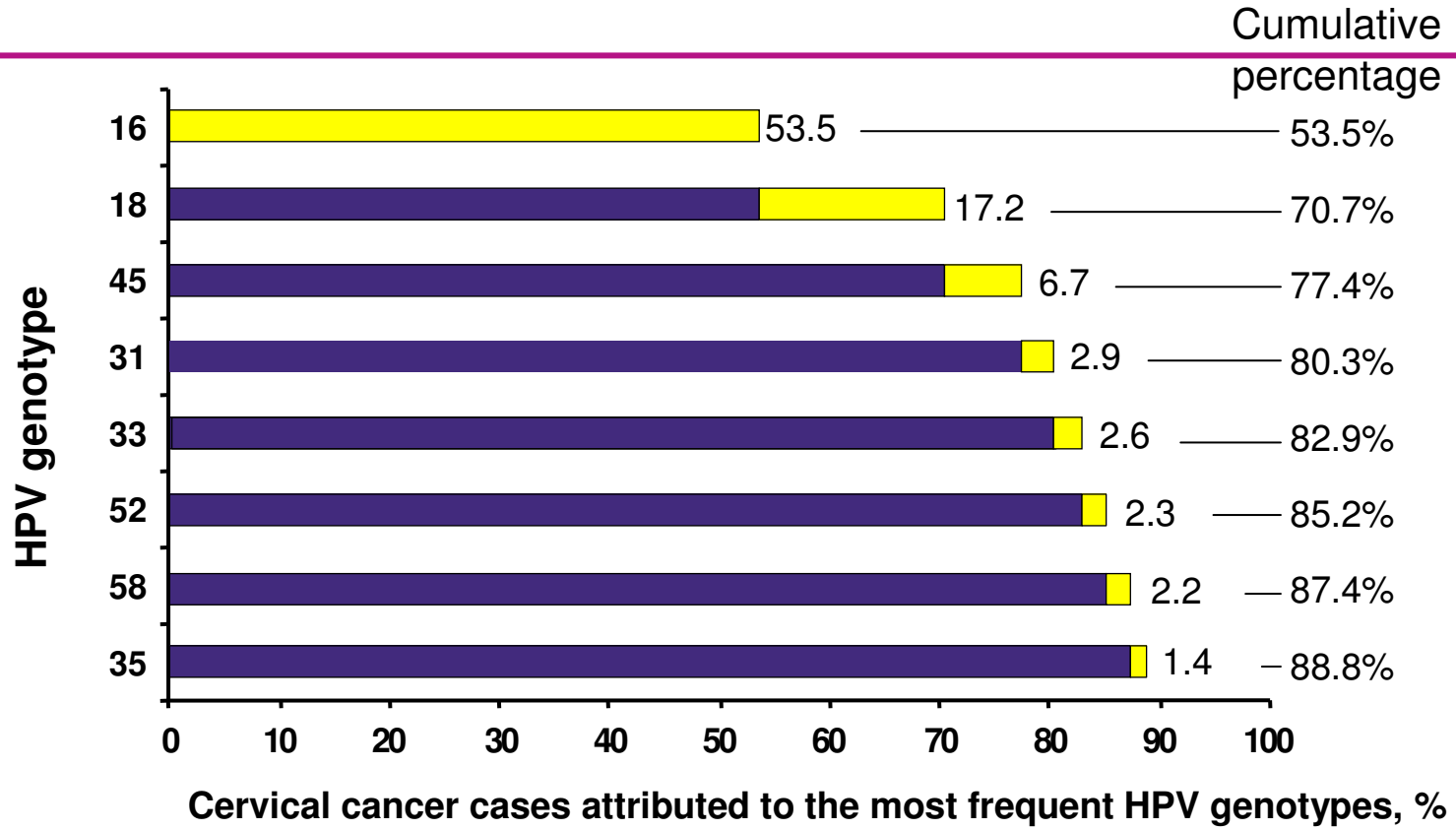
Differentiation – Dependent mRNA Expression and DNA Replication in an HPV-11 Laryngeal Papilloma

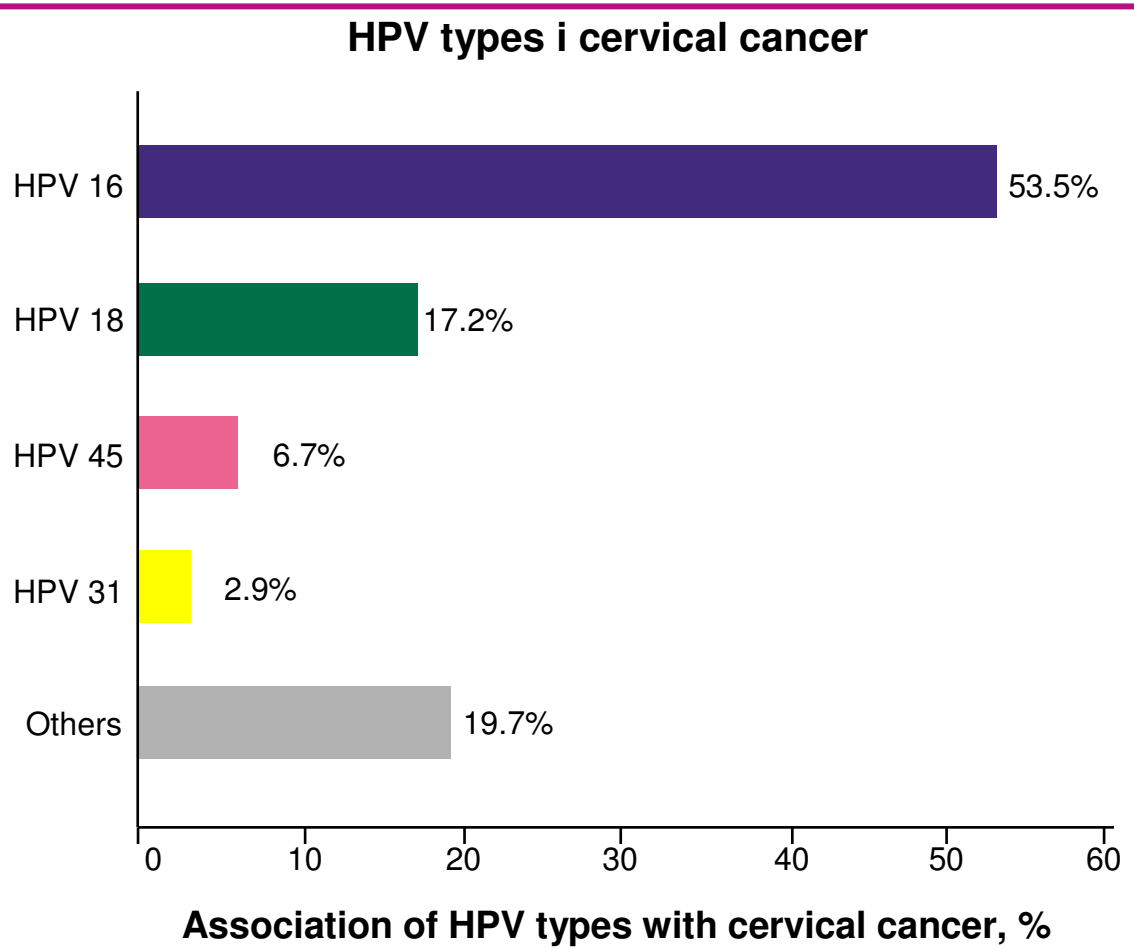


HPV types i cervical cancer globalt set



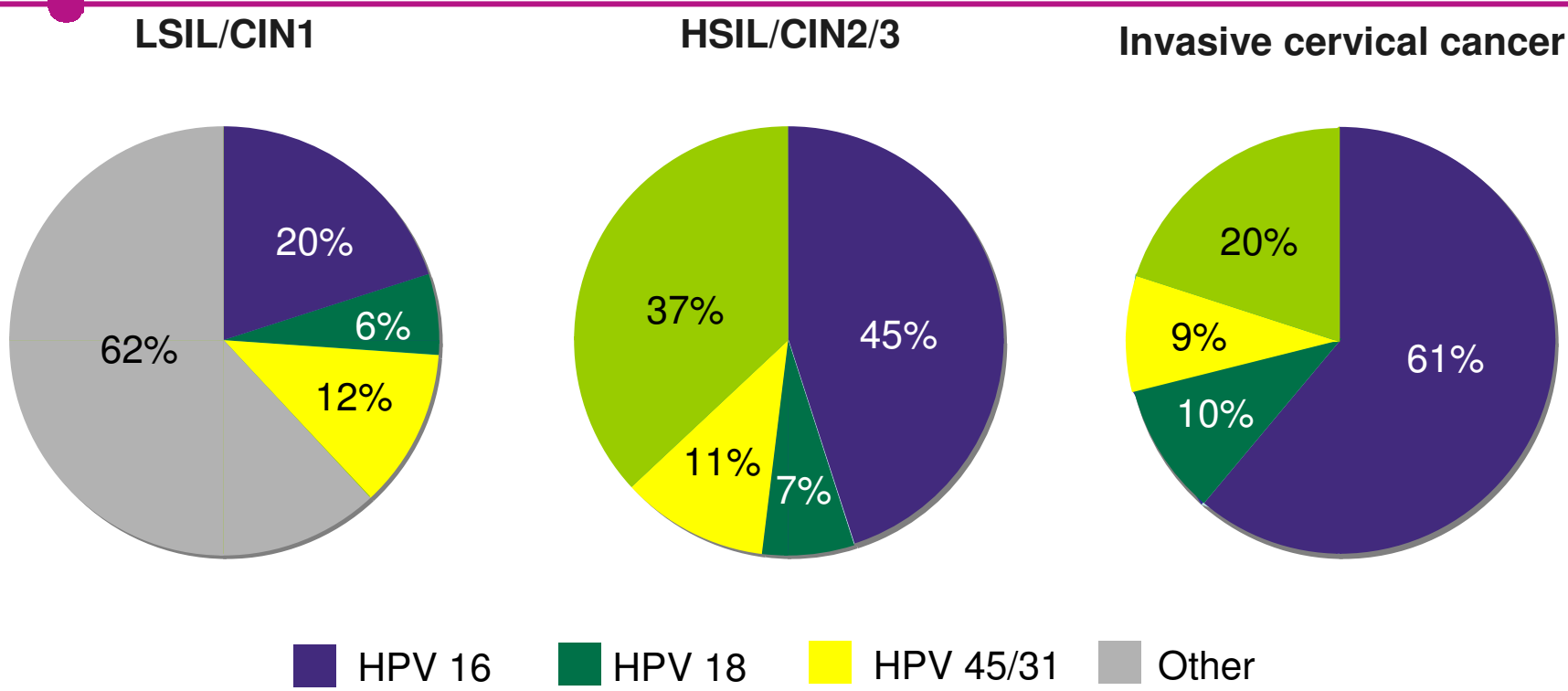
Muñoz N, *et al. Int J Cancer* 2004; **111:**278–285.

Associering af HPV typer med cervix cancer globalt set



Adapted from Muňoz N, *et al. Int J Cancer* 2004; **111:**278–285.

The most common HPV types according to grade of cervical lesion



Adapted from: http://www.who.int/hpvcentre/statistics; de Sanjose S *et al*. Presentation; 24th International Papilloma Conference, Beijing 2007.

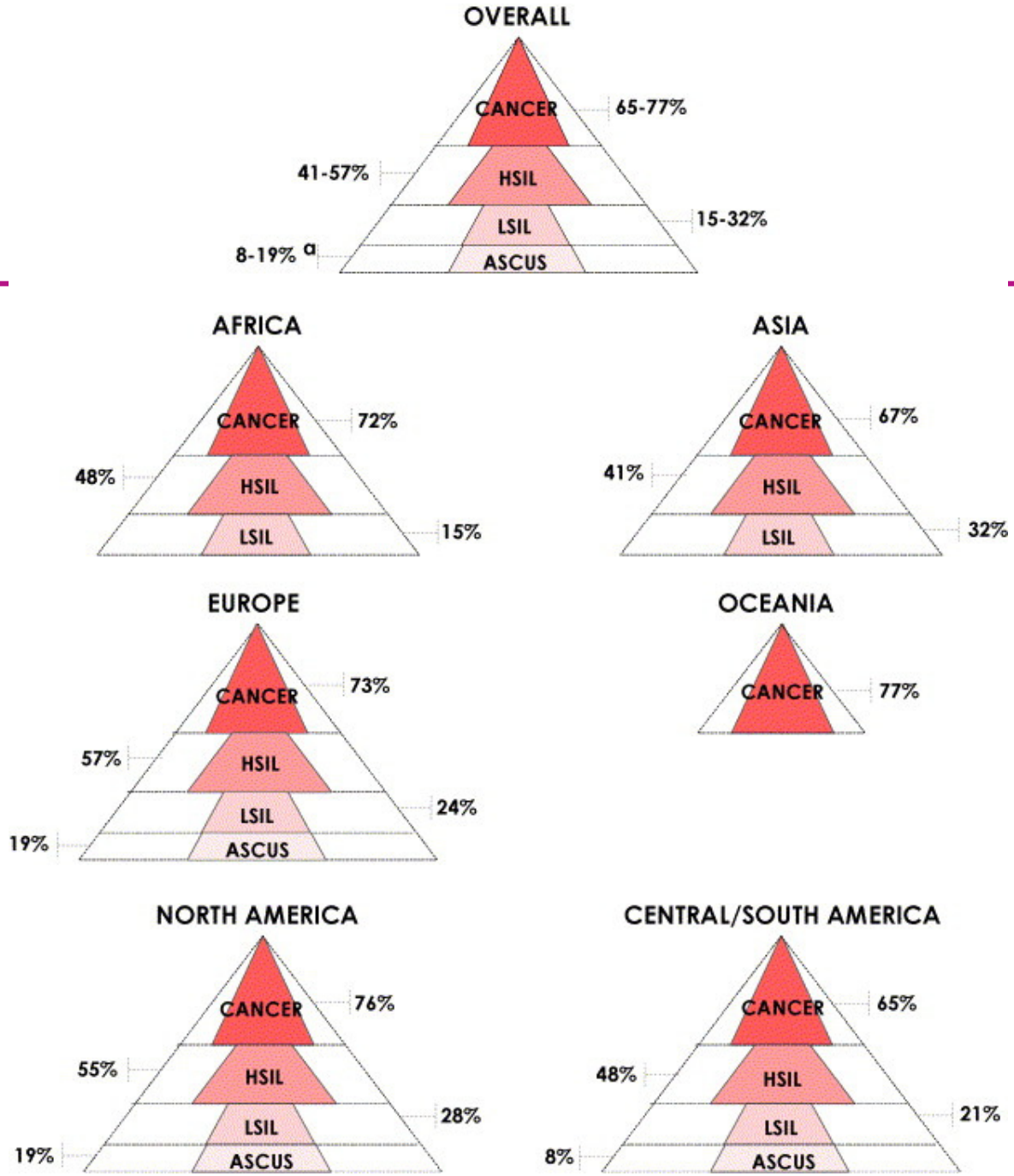
HPV infections relateret cancer i 2002

Site	Attrib to HPV, %	Developed countries			Developing countries		
		Total cancers	Attrib to HPV	All cancer, %	Total cancers	Attrib to HPV	All cancer, %
Cervix	100	83,400	83,400	1.7	409,400	409,400	7.0
Penis	40	5,200	2,100	0.0	21,100	8,400	0.1
Vulva/ Vagina	40	18,300	7,300	0.2	21,700	8,700	0.2
Anus	90	14,500	13,100	0.3	15,900	14,300	0.2
Mouth	3	91,100	2,700	0.1	183,000	5,500	0.1
Orophar.	12	24,400	2,900	0.1	27,700	3,300	0.1
All sites		**5,016,100**	**111,500**	**2.2**	**5,827,500**	**449,600**	**7.7**

Parkin, DM. *Int J Cancer* 2006; **118:**3030–3044.

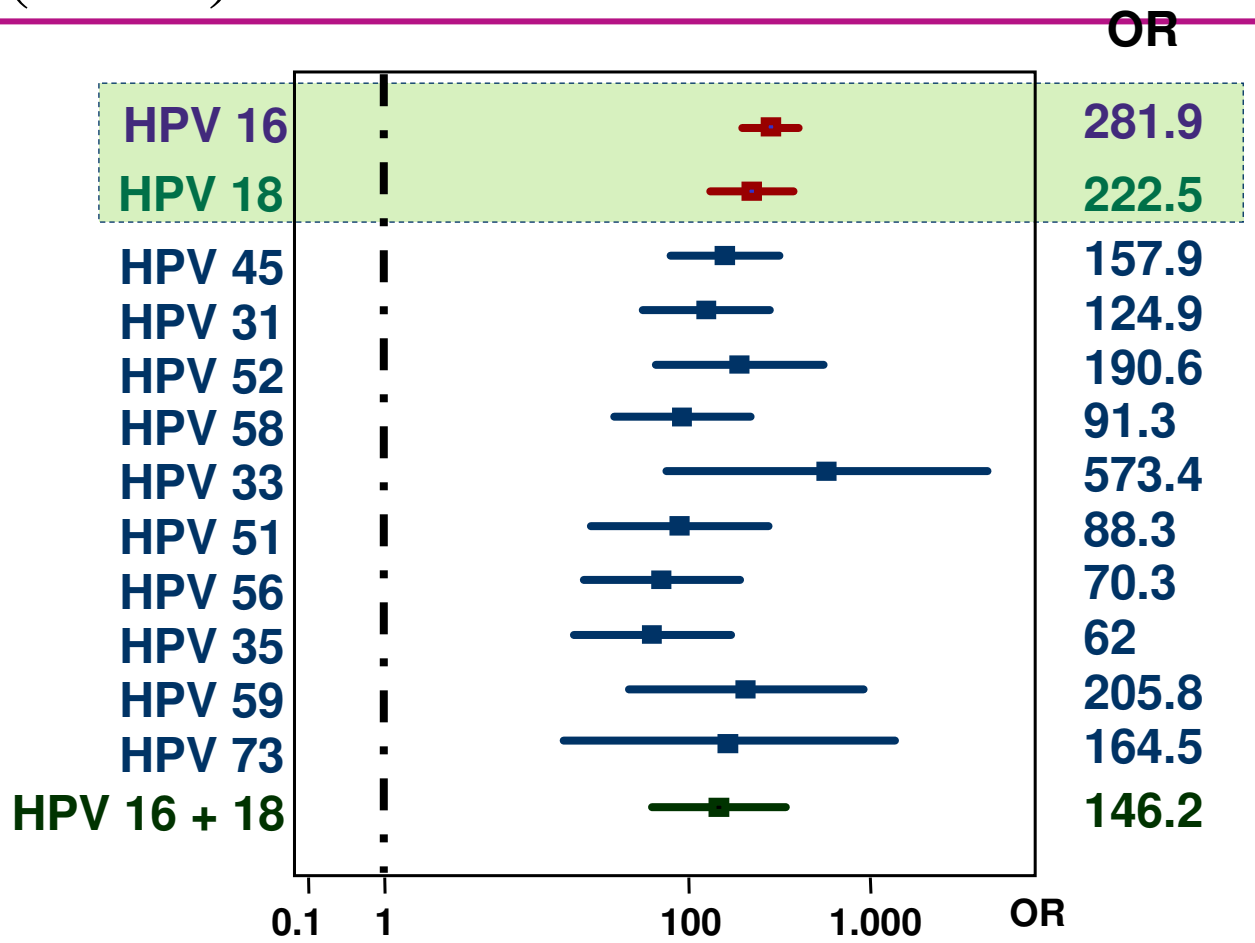
HPV-16/18 positive fraktion blandt cervical abnormalitet

Ref Clifford, Vaccine 2006



Risk of cervical cancer by HPV types: all International Agency for Research on Cancer (IARC) studies combined

- Type-specific odds ratio (OR) for cervical carcinoma (squamous cell and adenocarcinoma)
- Subjects with HPV DNA-negative results were used as the reference category
- ORs were adjusted by country and age group



1. Muñoz N, *et al. Vaccine* 2006; **24**(Suppl 3):S1–S10.
2. Muñoz N, *et al. N Engl J Med* 2003; **348:**518–527.

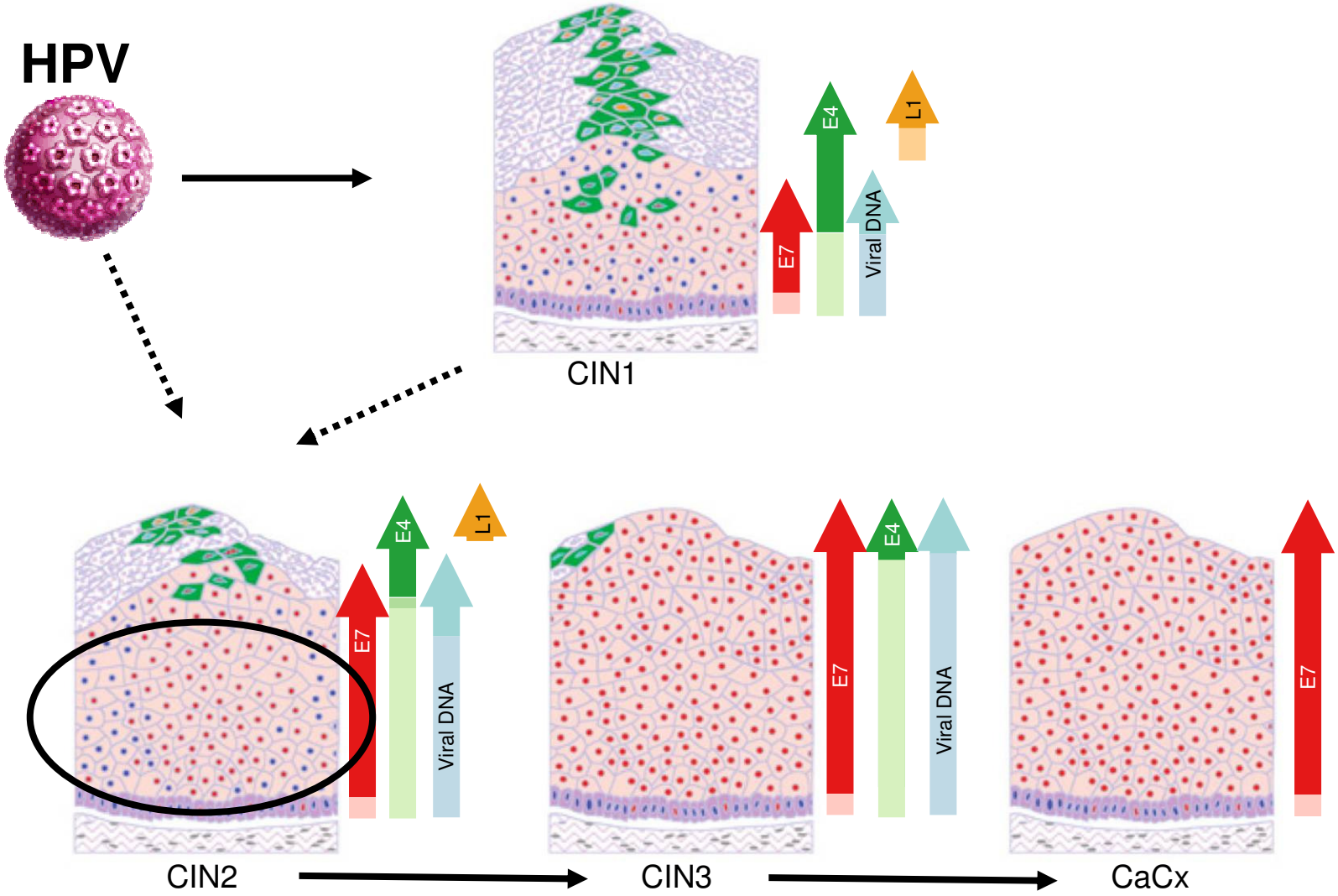
HPV pathogenese

- Protein products af de tidlige HPV genes E6 og E7 er ansvarlige for transformation og immortalisering af celler

- E6 og E7 interagerer med celle cyclus kontrol (p53 og Rb)

Münger K, *et al. J Virol* 2004; **78**:11451–11460.

HPV gene expression i persisterende infektioner og sygdoms-progression

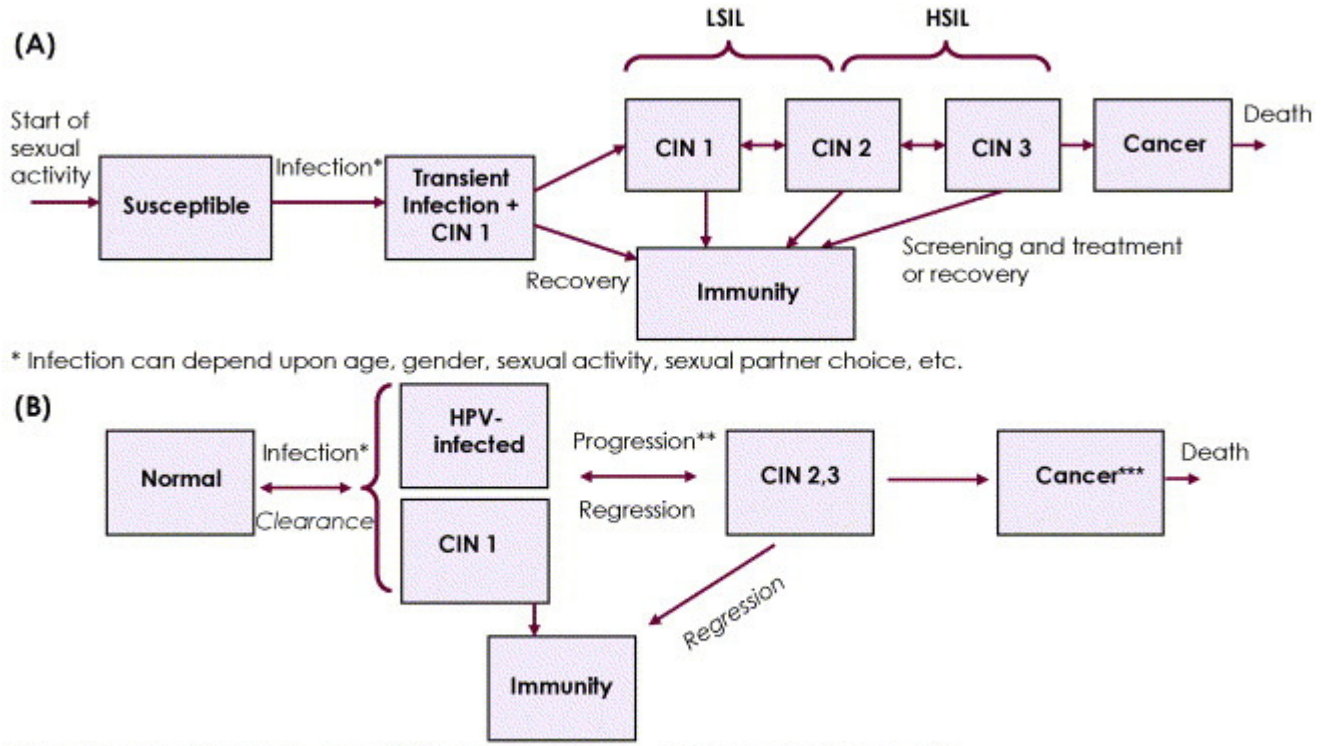


Doorbar J. *Clin Sci (Lond)* 2006; **110**:525–541.

Transmission og Sygdom

Ref. Goldie Vaccine 2006

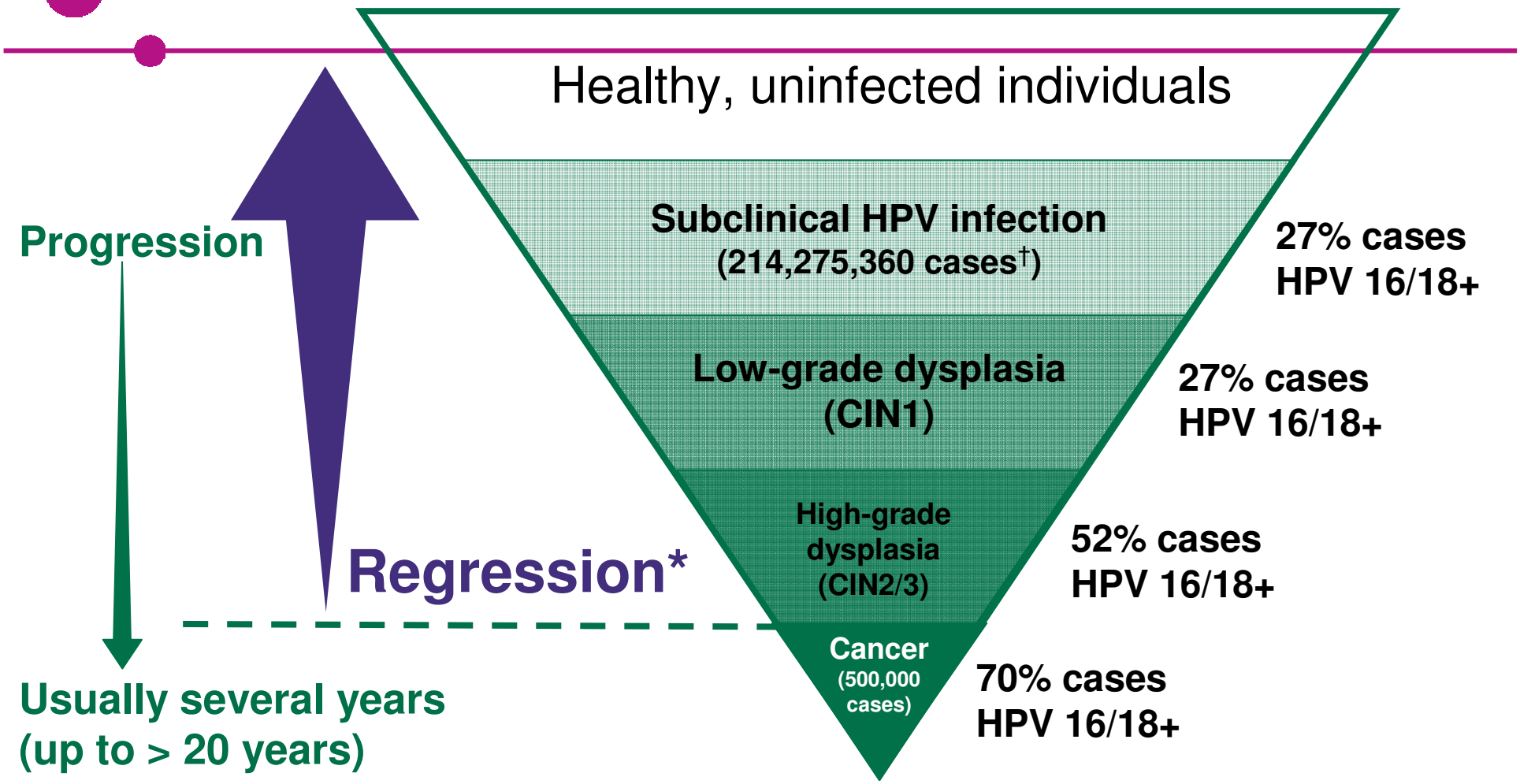
TWO SCHEMATIC REPRESENTATIONS OF THE NATURAL HISTORY OF HPV AND CERVICAL CANCER USED IN MATHEMATICAL MODELS



HPV pathogenese

- **Oftest benigne læsioner**:
- Vorter, Condylomas, Papillomas i naso-pharynx.
- **Maligne læsioner**:
- Carcinomas i genitalier, hud, larynx etc.
- **Epidermodysplasia verruciformis-EV**.

Risiko for progression til cervical cancer: *Global*



* Most cases of CIN spontaneously regress; less likely with higher grade lesions (cervical cancer does not regress).

† Estimated from total number of global females aged > 15 years.

Data available at: http://www.who.int/hpvcentre/en (accessed Sept 2008);

Castellsagué X, *et al. Vaccine* 2007; **25S**:C1–C26; Clifford GM, *et al. Lancet* 2005; **366:**991–998.

Cervical Adenocarcinoma

- Adenocarcinomas opstår fra glandular epithelia i endocervix mens squamous cell carcinomas opstår fra squamous epithelia i ectocervix[1]
- Adenocarcinomas udgør ca 15–25% af alle invasive cancere[2]
- Op til 30% diagnostiseres i kvinder < 35 år[2]
- Større risiko for recurrens sammenlignet med squamous cell carcinomas[3]
- Adenocarcinomas er hyppigst associeret med HPV 16 and 18 [4–7]

1. Cancer Research UK 2008. Accessed at http://www.cancerhelp.org.uk/help/default.asp?page=2758;
2. Parkin DM, *et al. Vaccine* 2006; **24**(Suppl 3):11-25;
3. Smith HO, *et al. Gynecol Oncol* 2000; **78**:97-105;
4. Altekruse SF, *et al. Am J Obstet Gynecol* 2003; **188**:657–663;
5. Smith JS, *et al. Int J Cancer* 2007; **121**:621–632;
6. Bosch XF, *et al. J Nat Cancer Inst* 1995; **87**:796–802;
7. de Sanjose S, *et al.* 24th International Papillomavirus Conference and Clinical Workshop; Beijing, 2007.

Cervix Cancer

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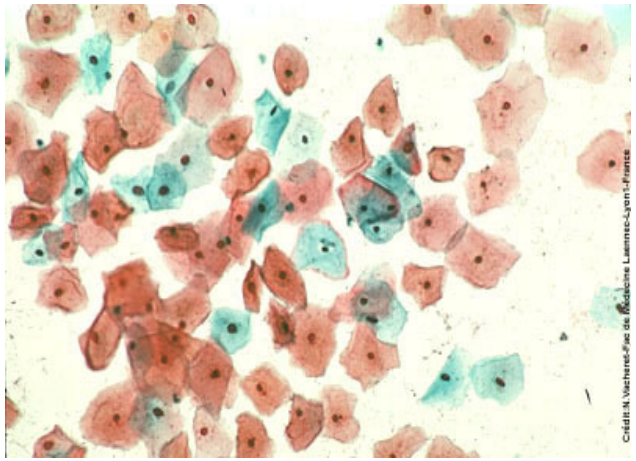

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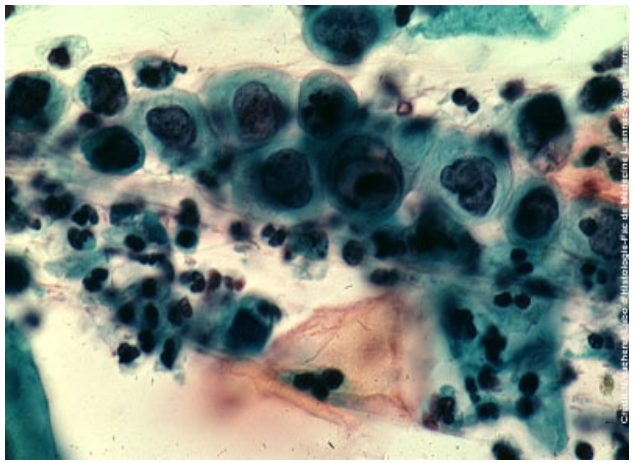
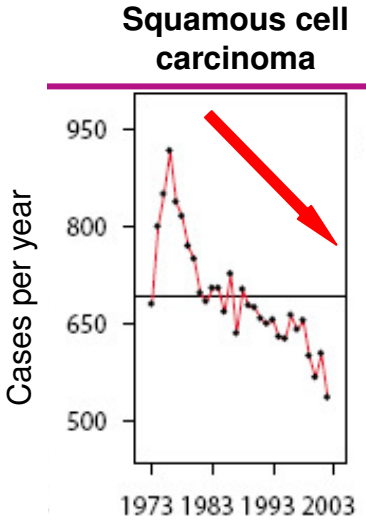
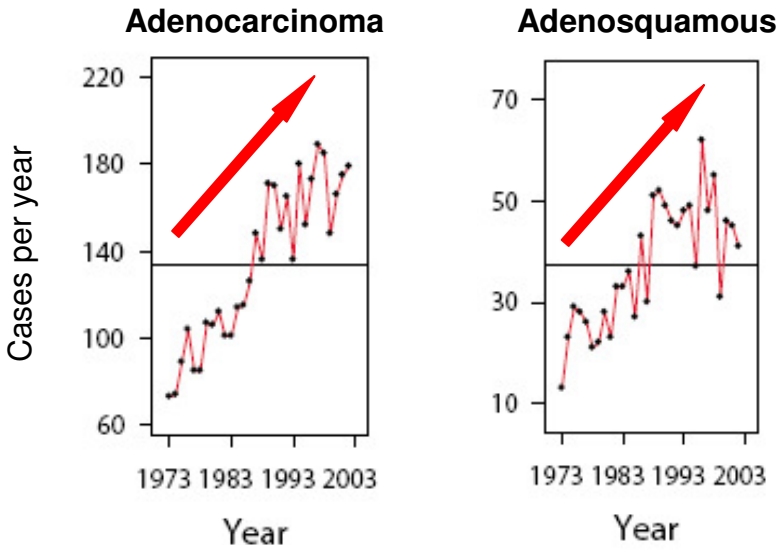


Nye tilfælde af adenocarcinoma er stigende

- Vinh-Hung *et al.* har vist et generelt fald i incidence af squamous cell carcinoma[1]

- Til gengæld er der en stigning i adenocarcinoma og adenosquamous carcinoma I den samme periode [1]





1. Vinh-Hung V, *et al. BMC Cancer* 2007; **7**:164–176.

Adenocarcinoma prognosis er dårlig

- Adenocarcinoma betragtes som en mere aggressiv tumour med en dårligere prognosis end squamous cell carcinoma fordi:
  - Den har tendens til at progrediere hurtigere[1]
- I et studie: 42% af 'rapid-onset' cervical cancer tilfælde var adenocarcinomas[1]
- Mere sandsynlig at den metastaserer tidligt I forløbet[2]

1. Hildesheim A, *et al. Am J Obstet Gynecol* 1999; **180**:571–577;
2. Krüger Kjaer S, *et al. Epidemiol Rev* 1993; **15**:486–498.

Væsentligste Risiko Faktorer

- HPV typer HPV-16, -18 eller andre high-risk types
- Persistent infektion
- Immunosuppression

Yderligere Risiko Faktorer

- Rygning?
- Multiparitet?
- Langtids oral contraceptives?
- Yderligere faktorer kan være, e.g.hormon status, immunstatus, genotoxic stoffers påvirkning?

HPV Prevalence og Persistence

- *Seroprevalence:*
- **HPV seroprevalence i USA, kvinder 20-29 år: 25-41%**
- **HPV seroprevalence i mænd >20%**

- *Persistence (CIN)*
- **CIN1:**

  spontan regression i 60% cases – ca.1% progress to cancer
- **CIN2/3:**

  spontan regression i 30-40% - >12% progress to cancer

Ref.Markowitz et al CDC recommendations 2007

HPV "clearance"

- Infektion med HPV 16, 18 og andre oncogenic typer har evnen til at persistere mere end low-risk HPV types[1–4]
- I kvinder 15–25 år er ~80% af HPV infektionerne transiente[5]
- I ældre kvinder er cervicale HPV infectioner oftere persisterende[6]
- Persistens med oncogene HPV typer er precursor for invasiv cervical cancer

Study (Country)	n	Average follow-up, years	Median duration of infection, months		
			Type 16	Type 18	Type 6
Ho, 1998 (USA)[1]	608	2.2	**11**	**12**	6
Muňoz, 2004 (Colombia)[2]	1610	4.1	**14**	**12**	-
Richardson, 2003 (Canada)[3]	621	1.8	**19**	**9**	6
Woodman, 2001 (UK)[4]	1075	2.4	**10**	**8**	9

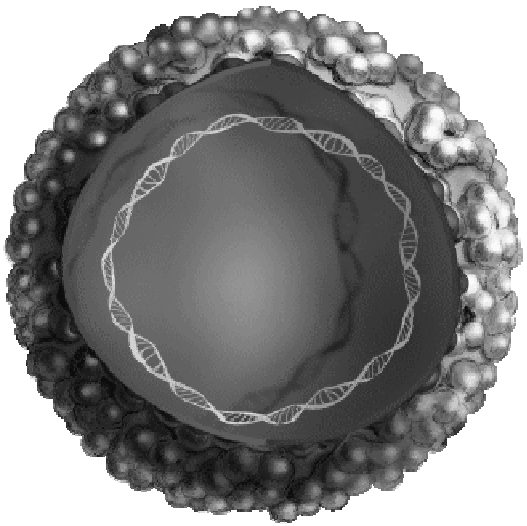
1. Ho GY, *et al. N Engl J Med* 1998; **338:**423–428; 2. Muňoz N. *J Infect Dis* 2004; **190:**2077–2087; 3. Richardson H. *Cancer Epidemiol Biomarkers Prevent* 2003; **12:**485–490; 4. Woodman CB. *Lancet* 2001; **357:**1831–1839. 5. Londesborough P. *Int J Cancer (Pred Oncol)* 1996; **69:**364–368; 6. Castle PE, *et al. J Infect Dis* 2005; **19:**1808–1816

HPV Vaccine

- L1 Capsids (VLP):
- HPV-6, 11, 16, 18 Gardasil™
  (license June 2006 for kvinder 9-26 år, Merk)
- HPV-16,18 Cervarix™
  (license 2007, Glaxo Smidt Kline)

Therapeutic vaccine: Under udvikling

Udvikling af virus-lignende partikler som vaccine antigens



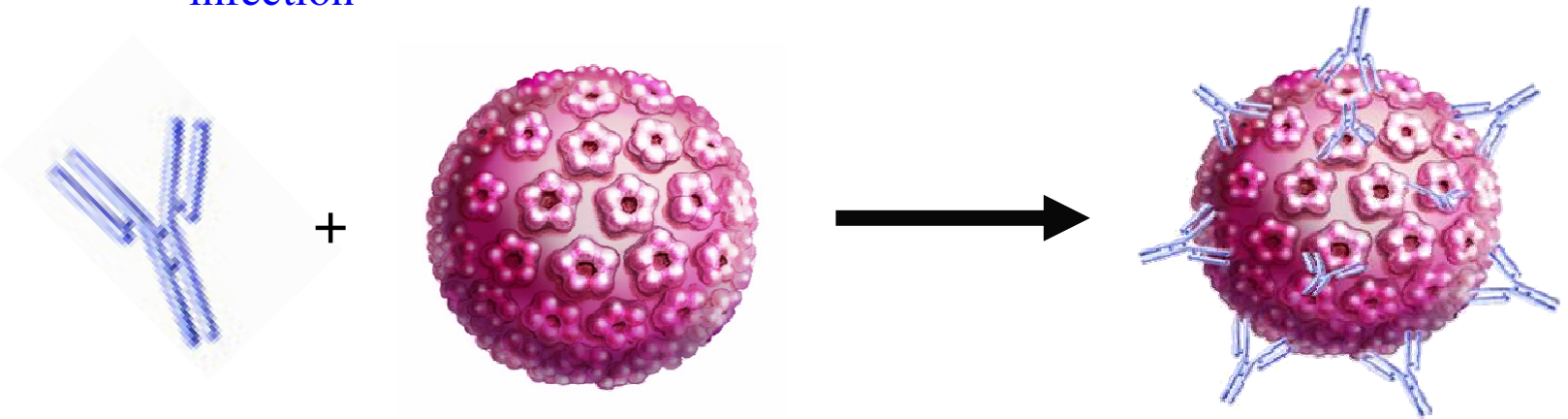
HPV–indeholdende double stranded DNA

'Empty' non-infectiøse virus-lignende partikler mimiker native virus

Stanley M, *et al. Vaccine* 2006; **24**(Suppl 3)**:**S106–S113.

Den immunologiske basis for cervical cancer vacciner

- Prophylaktisk vaccine focuserer på forebyggelse af infektion ved at forhindre virus I at komme ind i cellerne
- Animal modeller har demonstreret at serum neutraliserende antistoffer er årsag til væsentlig protektion[1–6]
  - Induction af neutraliserende antistoffer ved vaccination forhindrer infection[1–6]

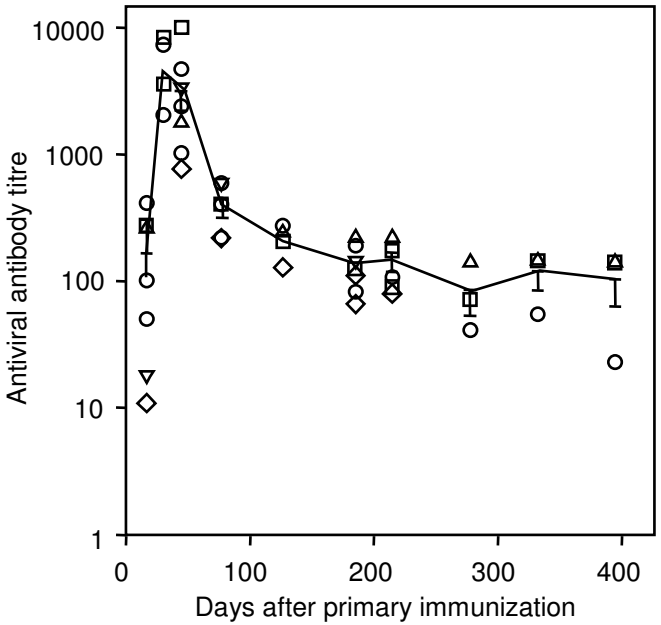


1. Breitburd F, *et al. J Virol* 1995; **69:**3959–3963; 2. Christensen ND, *et al. J Virol* 1996; **70:**960–965; 3. Suzich JA, *et al. Proc Natl Acad Sci USA* 1995; **92:**11553–11557; 4. Jansen KU, *et al. Vaccine* 1995; **13:**1509; 5. Kirnbauer R, *et al. Virology* 1996; **219:**37–44; 6. Lin Y-L, *et al. Virology* 1992; **187:**612–619.

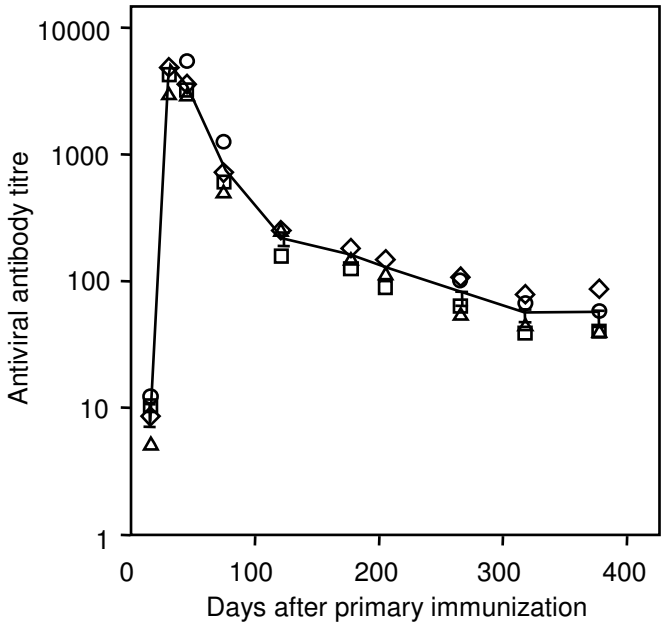
Immunisering med L1 VLPs kan føre til høje titre af virus-specifikke antistoffer I dyre modeller

Serum antistoffer i immuniserede kaniner[1]

CRPV L1 VLP



HPV 11 L1 VLP



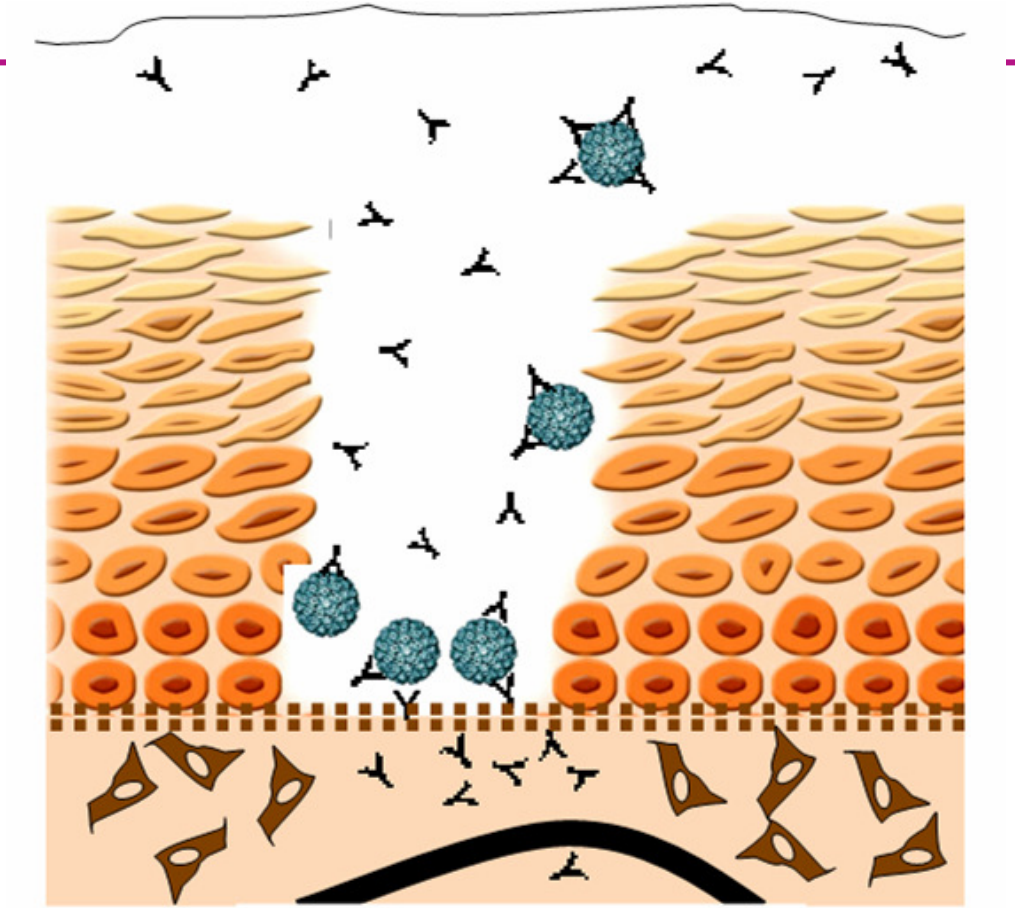
- ensartede data er beskrevet hos kvæg[2]
- Antistoffer med lavere titer er vist også i hunde[3]

1. Christensen ND, *et al. J Virol* 1996; **70:**960–965; 2. Kirnbauer R, *et al. Proc Natl Acad Sci USA* 1992; **89:**12180–12184;
3. Suzich JA, *et al. Proc Natl Acad Sci USA* 1995; **92:**11553–11557.

Hvorledes IM injektion af VLP vaccine forebygger mucosa infection i cervix

Transudated Abs i mucus*

Exudated Abs i traumet



Cervical mucus

Cervical epithelium

Basement membrane

Submucosa

* **VLP-specific IgG i cervical mucus ~10-fold lavere end i serum efter intramuscular (IM) vaccination.[2]**

1. Munoz N, *et al.* Chapter 1: HPV in the etiology of human cancer. *Vaccine* 2006; 24(Suppl 3)**:**S1–S10;
2. Nardelli-Haefliger D. *et al. J Natl Cancer Inst.* 2003; **95:**1128–1137; 3. Giannini S, *et al. Vaccine* 2006; **24:**5937–5949;
4. Poncelet S. *et al.* Abstract. Annual Meeting of the European Society for Pediatric Infectious Diseases. May 2–4, Porto, Portugal. 2007.

Summary of quadrivalent vaccine efficacy

Ref Marcowitz 2007

	Vaccine		Placebo		%Efficacy
	No	*cases*	*No*	*cases*	
HPV 16 or 18 related CIN2/3 or AIS					
Protocol 006	756	0	750	12	100
Protocol 007	231	0	230	1	100
Protocol 013	2200	0	2222	10	100
Protocal 015	5301	0	5258	21	100
Combined protocols	**9497**	**0**	**9460**	**53**	**100**
HPV 6,11,16,18 related CIN1,CIN2/3 or AIS					
Protocol 007	235	0	233	3	100
Protocol 013	2240	0	2258	37	100
Protocal 015	5383	4	5370	43	90
Combined protocols	**7858**	**4**	**7861**	**83**	**95**
HPV6,11,16,18 related warts					
Protocol 007	235	0	233	3	100
Protocol 013	2261	0	2279	29	100
Protocal 015	5401	1	5387	59	98
Combined protocols	**7897**	**1**	**7899**	**91**	**98**

Cervarix™ giver vedvarende beskyttelse mod cytological abnormalitet og CIN for HPV types up to 6.4 years

Kombineret analyse af initial efficacy og follow-up[1]

Estimated prevalence HPV 16/18, %	Endpoint	*Cervarix*™ N = 505	Al(OH)$_3$ N = 497	Vaccine efficacy	
		n	n	%	95% CI
20–30[2]	≥ ASCUS	118	162	**35**	18–50
25–30[2]	≥ LSIL	62	93	**39**	16–57
25–30[2]	CIN1+	20	38	**50**	12–73
50[3]	CIN2+	5	17	**72**	21–92

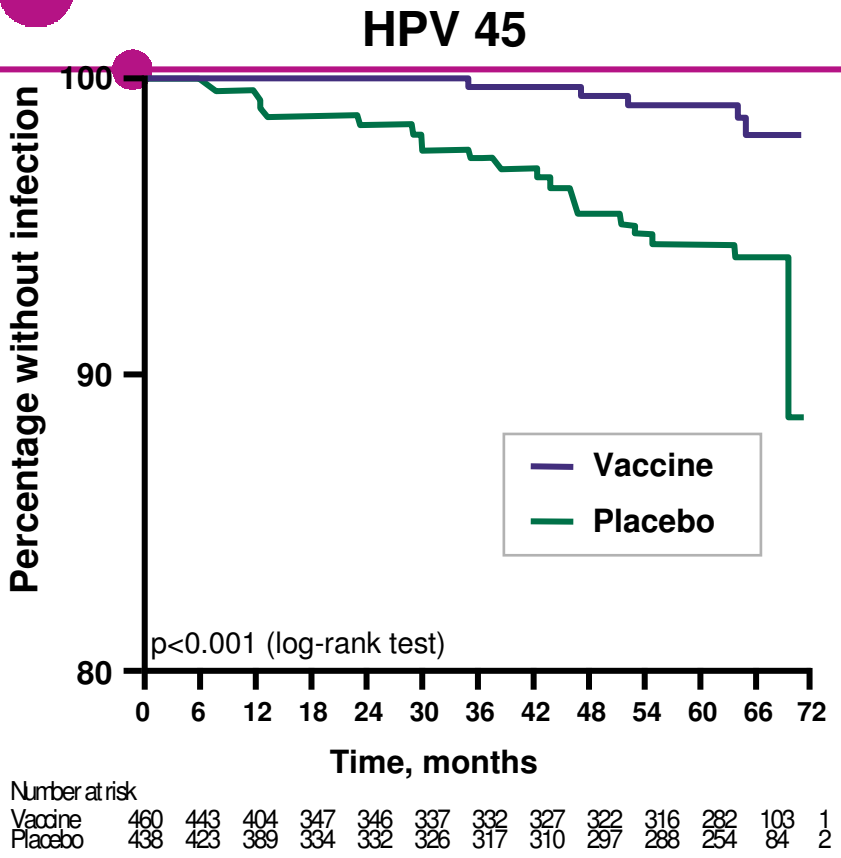
Uafhængigt af HPV DNA status

Cervical samples; descriptive, conditional exact method; ITT analysis

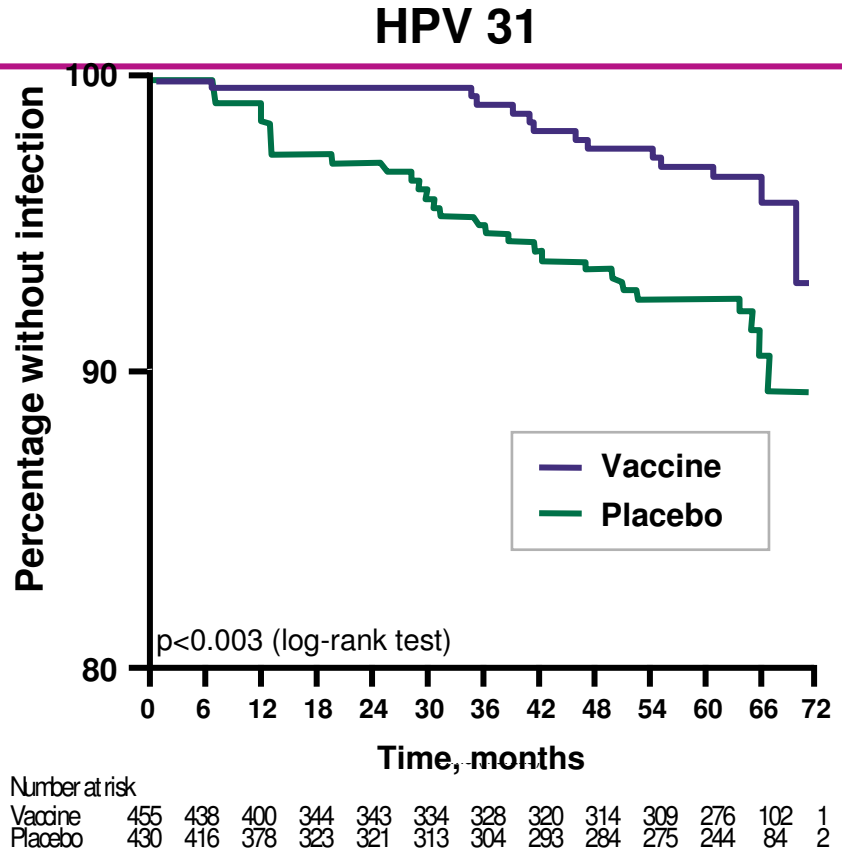
N = antal subjects inkluderet I hver gruppe; n = antal subjects reporterende mindst en episode I hver gruppe

1. Harper D, SGO. Tampa, Florida, March 9–12, 2008; Presentation; 2. Clifford GM, *et al. Cancer Epi Biom Prev* 2005; **14**:1157–1164; 3. Muñoz N, *et al. N Engl J Med* 2003; **348**:518–527.

Cervarix™ (HPV16/18) giver markant "efficacy" mod infection with HPV types 45 and 31



Vaccine efficacy: 78% (range: 39–93%)



Vaccine efficacy: 60% (range:20–81%)

HPV 45 and 31: 3. og 4. mest almindelige HPV typer fundet i cervical cancer globalt[3]

Total cohort; cervical samples only; Cox regression model

Adapted from 1. Gall S. AACR. Los Angeles, US, 14–18 April 2007, presentation; and 2. GSK data on file, 2008. 3. Muñoz N, *et al. Int J Cancer* 2004; **111**:278–285

Cervarix™ Kryds-protektions "efficacy" i analyse af Phase III data

Yderligere protektion mod 6-mdr persistens af infektion i Phase III PATRICIA studiet*

HPV type	Vaccine group (cases)	Control group (cases)	Vaccine efficacy, % (97.9% CI)	*p*
HPV 45	10/6,724	25/6,747	**59.9** (2.6–85.2)	0.0165
HPV 31	47/6,615	74/6,667	**36.1** (0.5–59.5)	0.0173

Broad protection against 12-month persistent infections in the Phase III PATRICIA study*

12 combined oncogenic types (excluding 16/18)	100	137	**27.1** (0.5–46.8)	0.0174

* Efficacy I den totale vaccinerede cohorte.

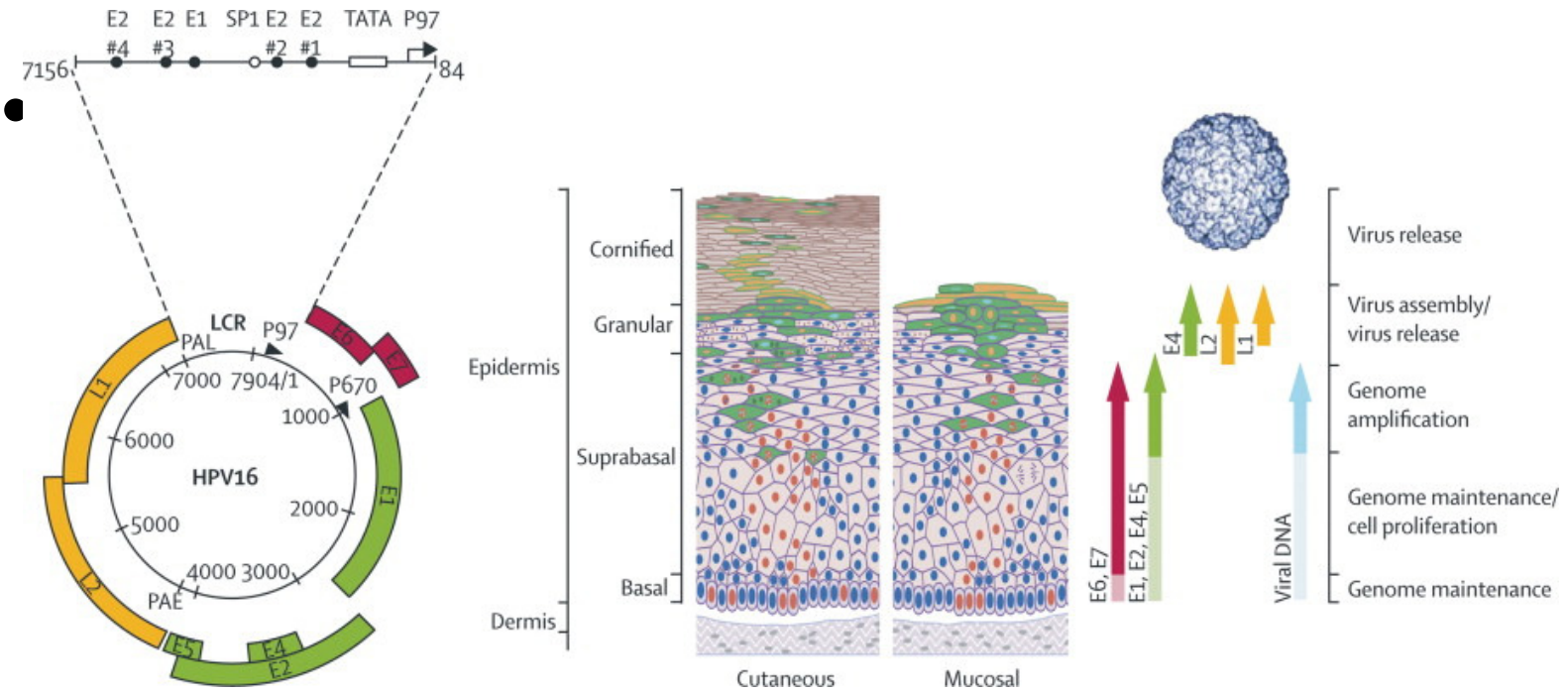
Paavonen J, *et al. Lancet* 2007; **369**:2161–2170.

Frentidens udfordringer

- Opfølgning af vaccinerede
- Screening-metoder?
- Hvad med multiple infektioner?
- Udvikling af nye vacciner
- Udvikling af nye behandling til HPV inficerede

HPV tests baseret på HPV DNA og expression (RNA eller protein)

Ref. Schiffman et al Lancet 370,p890,2007



Tests for HPV

- **1. PCR-Simple DNA based**
  - Type specifikke primers
- **2. PCR-DNA based – Generelle primers**
  -Score positive/negative
  -Typning af positive samples
  bl.a. ved sekventering
- **3. Hybrid capture**
- **4. LiPA**
- **5. PCR-DNA : Multiplex detection system**
- **6. In situ hybridization-uden amplification**
- **7. RT-PCR – RNA based**
- **8. Microchip arrays**

Vel kendte tests for HPV: Fordele og ulemper

- **PCR-DNA baseret**
  - Type specifikke primere

- **PCR-DNA baseret**
  – Generelle primere:
  1.Scores som positive/negative
  2.Typing af positive samples

- **Hybrid capture**

- **10-100 kopier**
  Primers i E6 eller E7

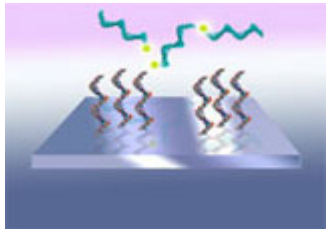
- **10-100 kopier**
  Primers i L1 eller E1

- **300-500 kopier**
  Hybridization-Sekventering for typning

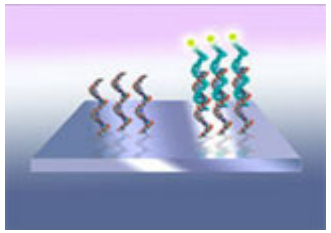
Vel kendte tests for HPV: Fordele og ulemper

- **4. LiPA**
- **5. PCR-DNA : Luminex detektion system**
- **6. In situ hybridization-Ingen amplifikation**
- **7. RT-PCR – RNA baseret HPV-proofer; APTIMA**
- **8. Microchip arrays**

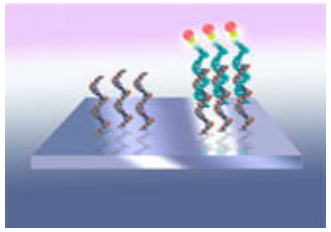
- **Amplificerer små sekvenses 69 bp god sensitivitet og specificitet**
- **Meget specifik og sensitive – god til store screeninger**
- **Ikke akceptabel sensitivity til diagnosis men informativ for pathologi**
- **Måler HPV infektion i et aktivt stadium**
- **Fremtidens design til stort antal prøver og til individuel diagnose**



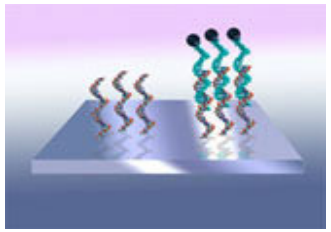
DNA-prøven, mærkes med biotin, og tilsættes microarray



Det biotin-mærket DNA bindes specifikt til deres komplementære sonder på microarray.

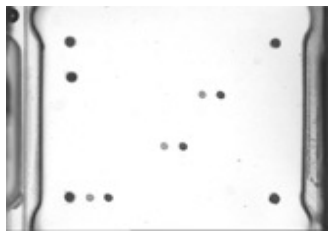


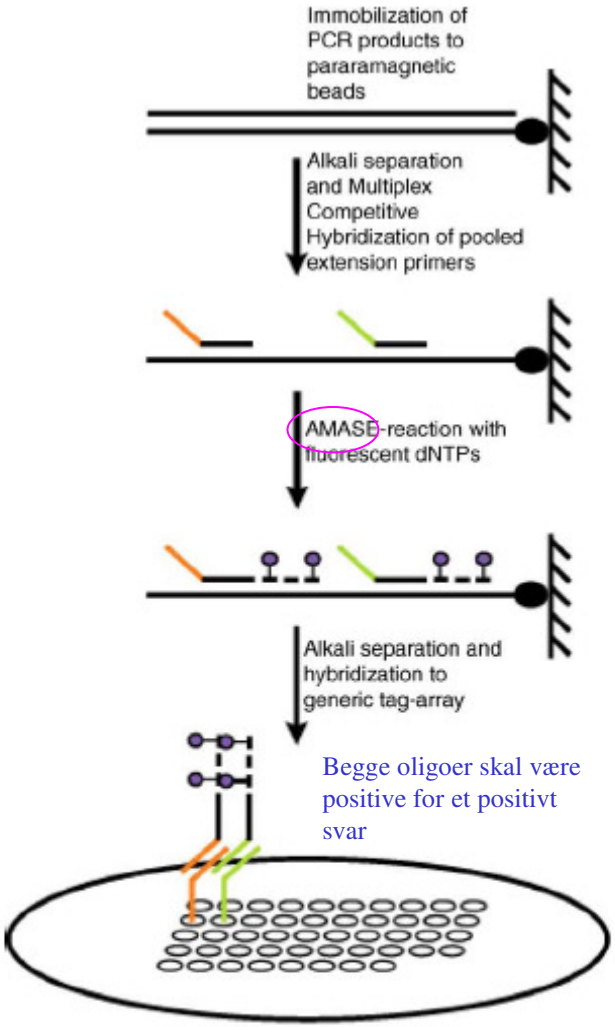
Streptavidin-peroxidase tilsættes og binder sig til biotin på DNA-prøven.



Fremkaldes med tetramethylbenzidina (TMB)
som udfældes , hvor DNA prøben er bundet

BN 2009





(A)

6-1	6-2	42-1	11-1	11-2	42-2	16-1	16-2	43-1
18-1	18-2	43-2	31-1	31-2	52-1	33-1	33-2	52-2	40-1
40-2	34-1	45-1	45-2	34-2	53-1	53-2	44-1	73-1	73-2
44-2	35-1	35-2	66-1	66-2	39-1	39-2	58-1	58-2	59-1
59-2	51-1	51-2	68-1	68-2	69-1	69-2			

(B)

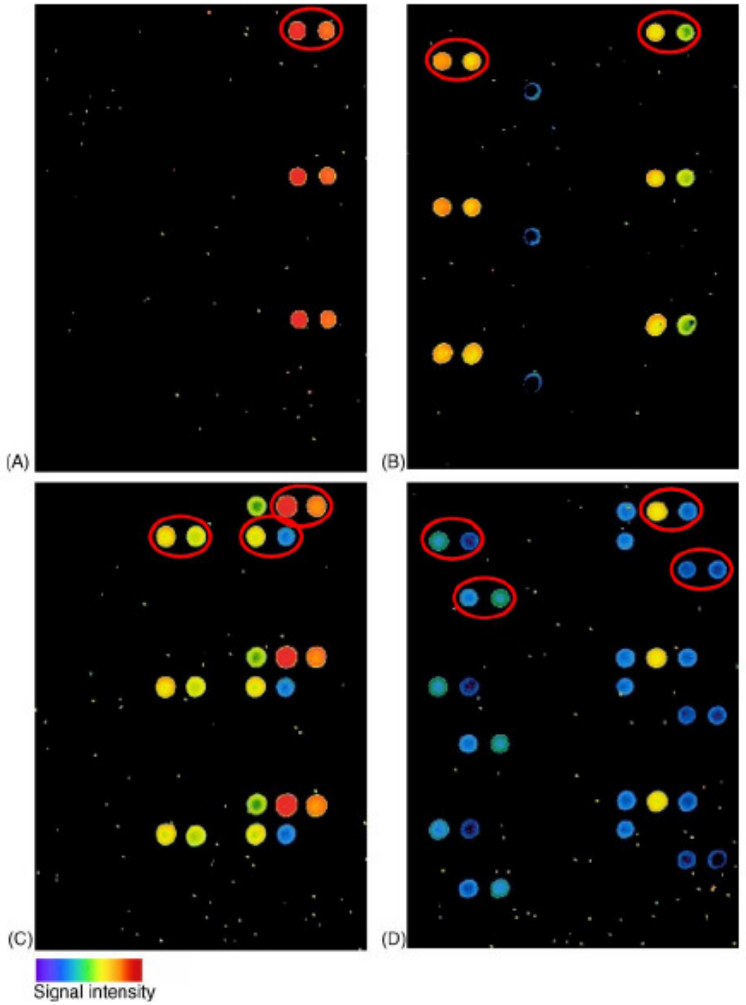


Fig. 2. Microarray images of a single, double, triple and quadruple infection, respectively (A–D). The oligonucleotides are spotted in triplicates and the positive signals in the first replicate are indicated with ovals. Sample 30 (for sample numbers see Table 2) has signals in both spots for HPV 16 and thus contains a type 16 infection (A). Sample 78 has infections of types 16 and 18 (B). Sample 89 has positive signals from both oligonucleotides for types 16, 31 and 33 and has infections of these genotypes (C). HPV types 16, 35, 18 and 73 are present in sample 90 (D). We observed that the signal for HPV 16 oligonucleotide 2 was considerably lower than oligonucleotide 1 (C and D). Sequencing confirmed that we had made a mistake at one base at the primer design (an A–G in base 13 of 20). This exemplifies the difficulties in discriminatory hybridization with lower signals for one probe observed in (C) and (D) as well as a high background signal in oligonucleotide 2 of HPV 42. However, it should be mentioned that despite this mistake HPV 16 could be scored. This is a strong indication that HPV types with intratypic variations can be scored by MUCH-AMASE but the quantification may not be correct. Note that the results in (A) and (B) are with the corrected oligonucleotide no. 2 for HPV16. In addition, the signal intensities for the first and the second spots, for each HPV type, may differ depending on sequence context.

LiPA

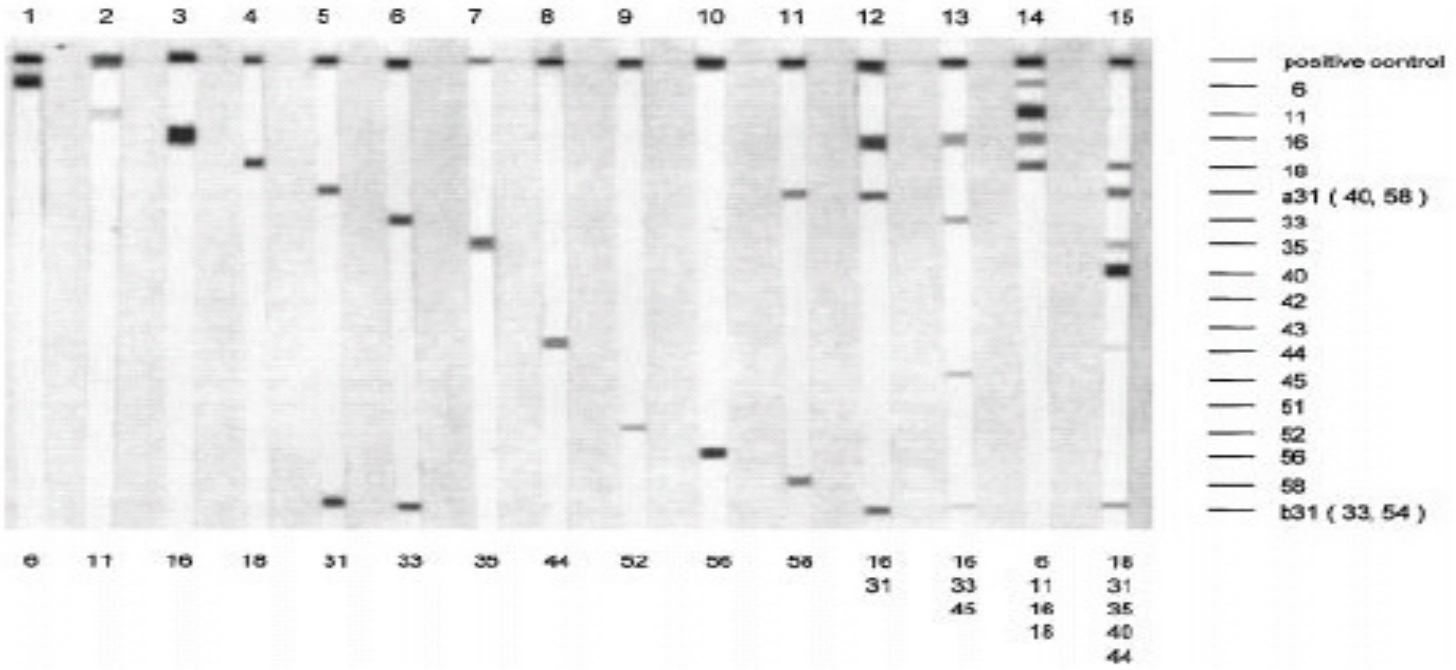


Figure 1. HPV genotyping of SPF amplimers by line HPV probe assay (HPV-LiPA). The grid on the right indicates the positions of different HPV probes. Positive control indicates the conjugate control line. The probe a31 reacts with products after PCR amplification of HPV 31 as well as HPV 40 and HPV 58. HPV 40 and HPV 58 have specific probes in a different location on the strip. Presence of HPV 31 can be identified when an additional band in location b31 is present. The numbers above the LiPA strips indicate consecutive samples. The numbers below the strips indicate HPV type(s) detected in the assay. In the last four strips multiple HPV types are detected.

Multiple infektioner

Ref. Meilhede et al 2009

APMIS

ACTA PATHOLOGICA, MICROBIOLOGICA ET IMMUNOLOGICA SCANDINAVICA

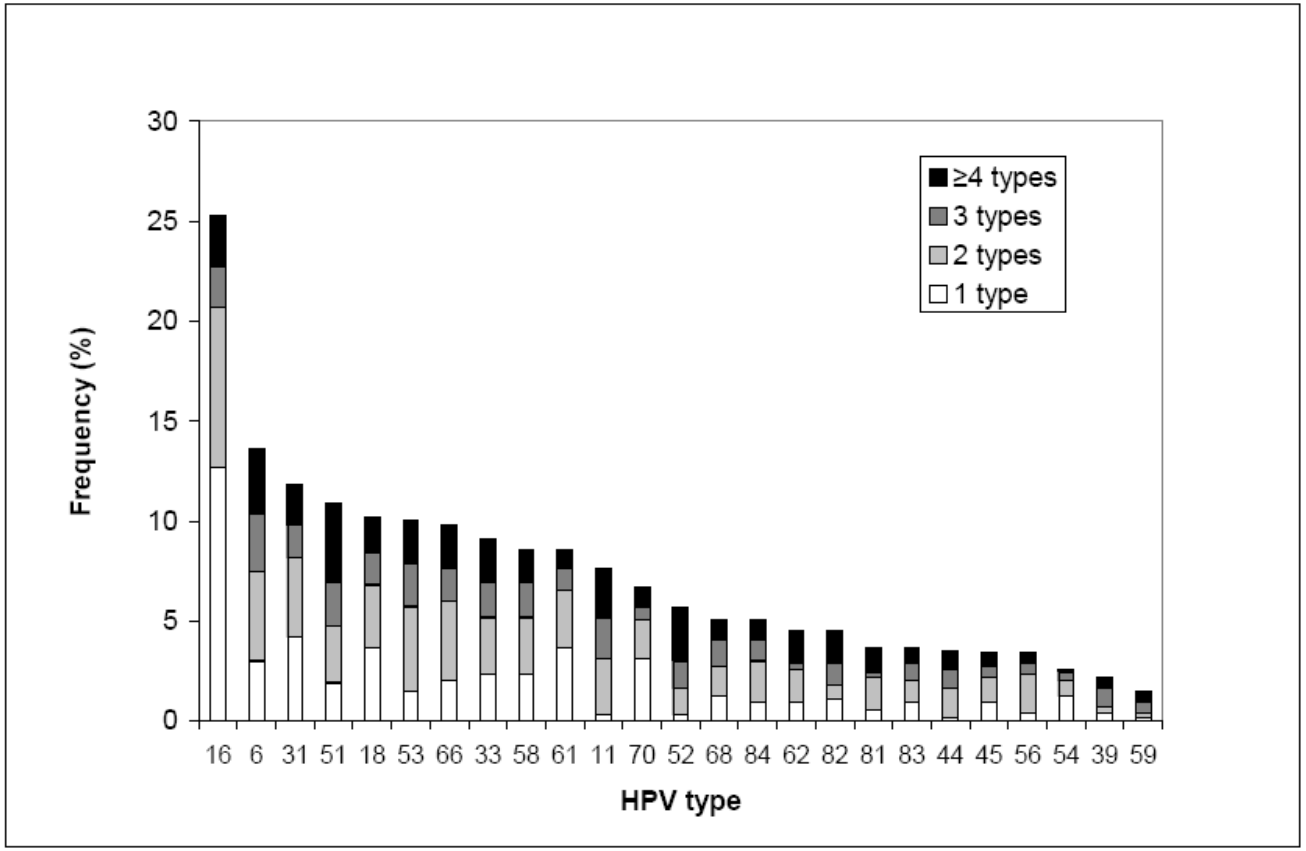


Figure 1. HPV type distribution among 735 HPV positive cervical specimens.
(49% var multiple infektioner)

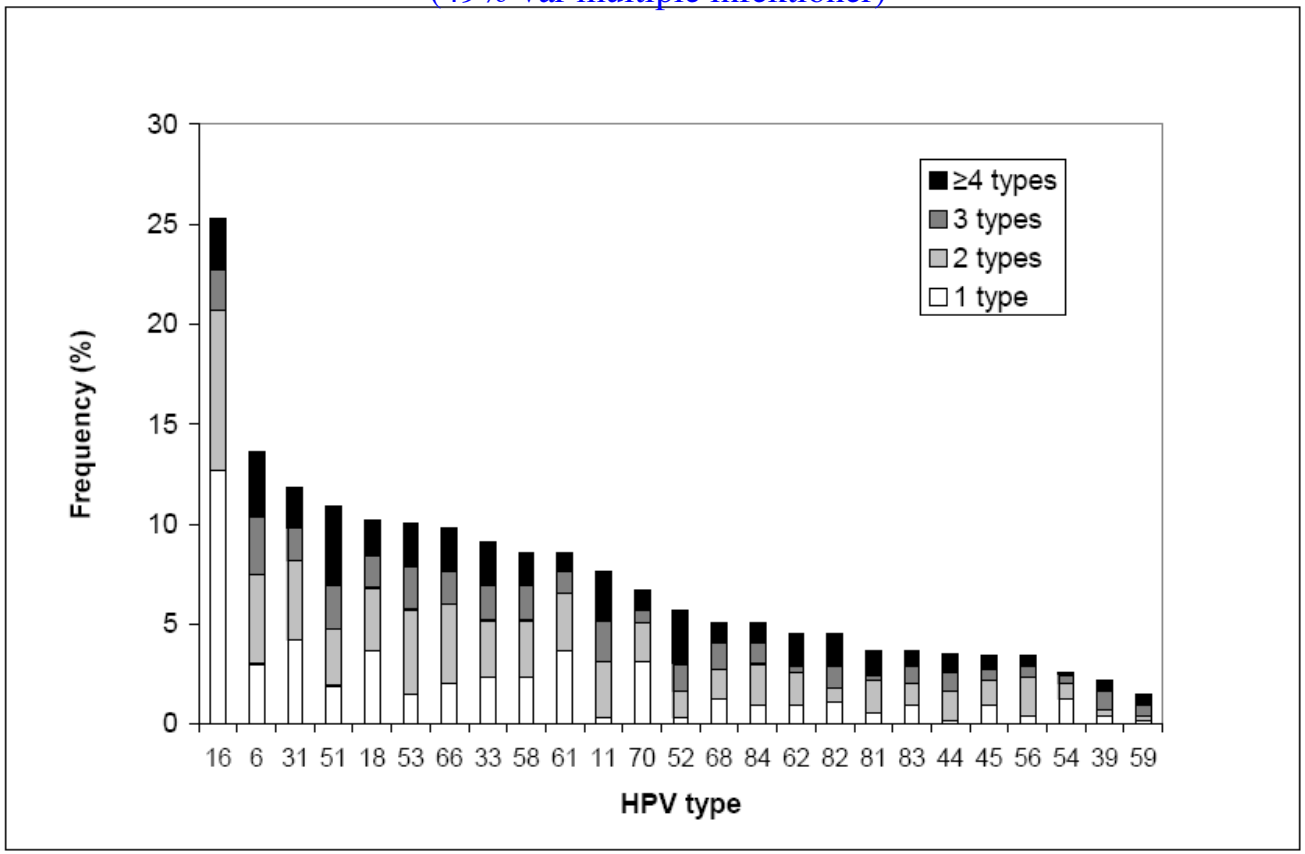
PCR med generelle MY09/11 primere og efterfølgende sekventering

Multiple infektioner

Ref. Meilhede et al 2009

Figure 1. HPV type distribution among 735 HPV positive cervical specimens.

(49% var multiple infektioner)



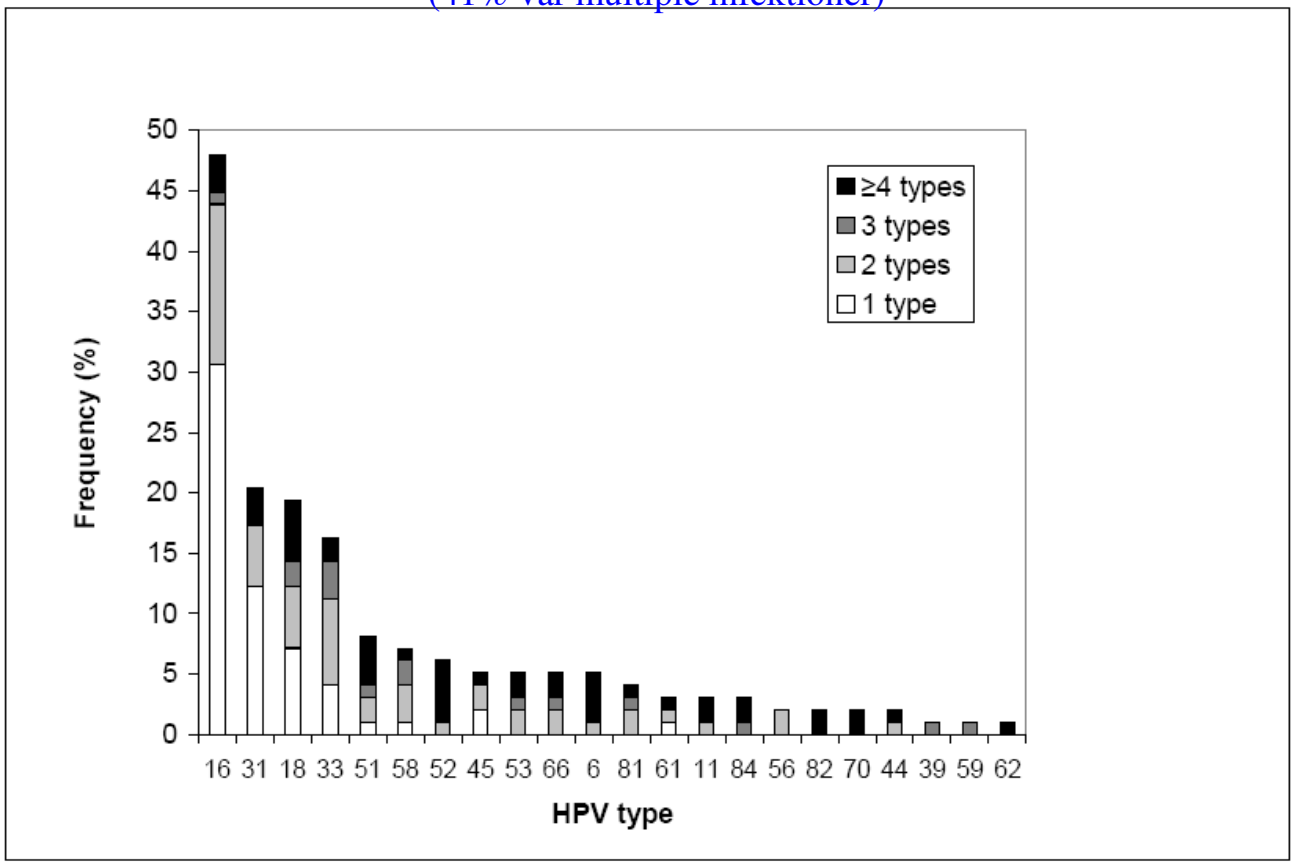
PCR med generelle MY09/11 primere og efterfølgende sekventering

Multiple infektioner

Ref. Meilhede et al 2009

Figure 3. HPV type distribution among 98 HPV positive cervical specimens from women diagnosed with CIN2+.

(41% var multiple infektioner)



Diagnostisk Fremtid!

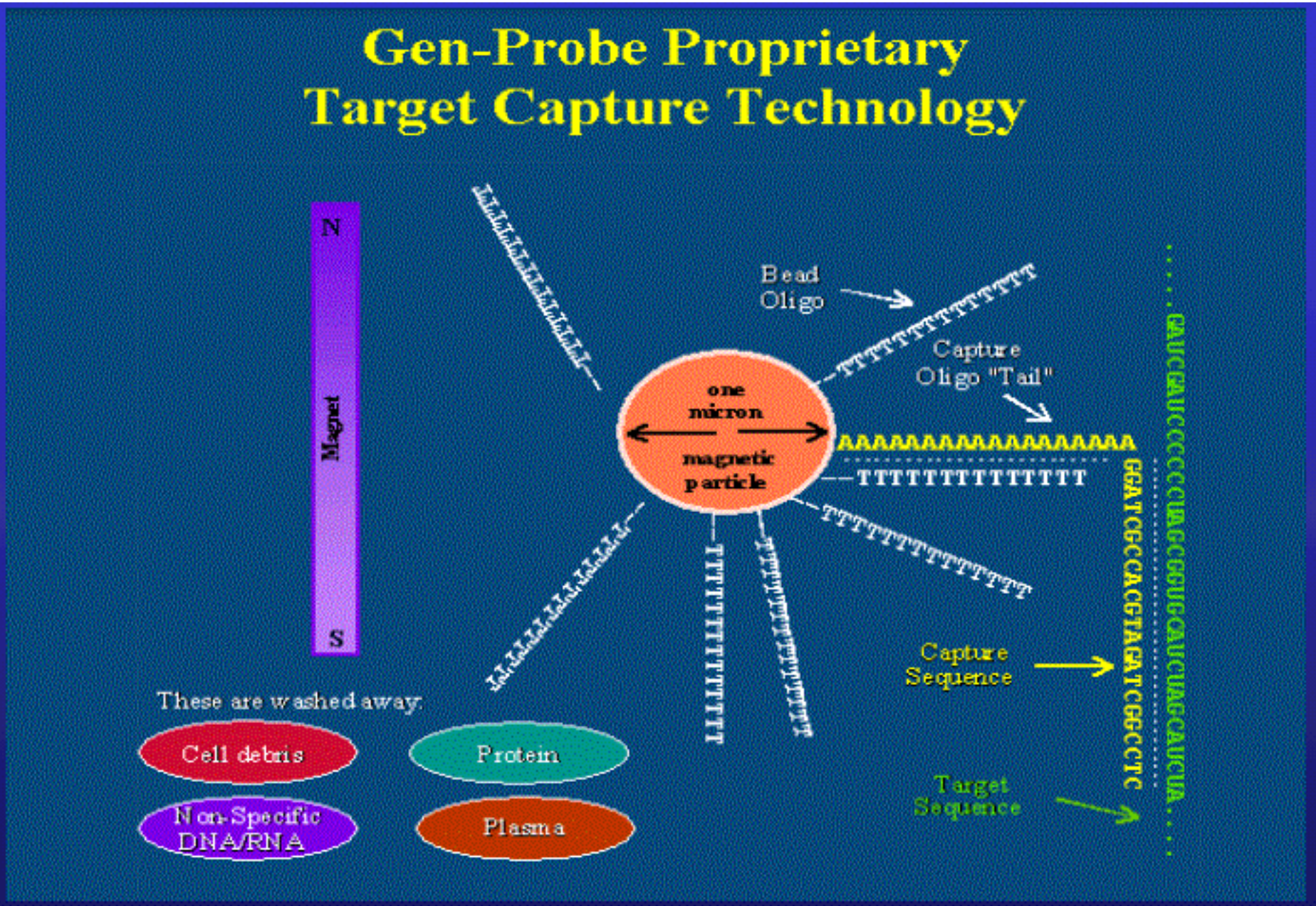
- Methoder vil ændres hurtigt

  mens
- Implementering og godkendelse fra myndigheder tager tid

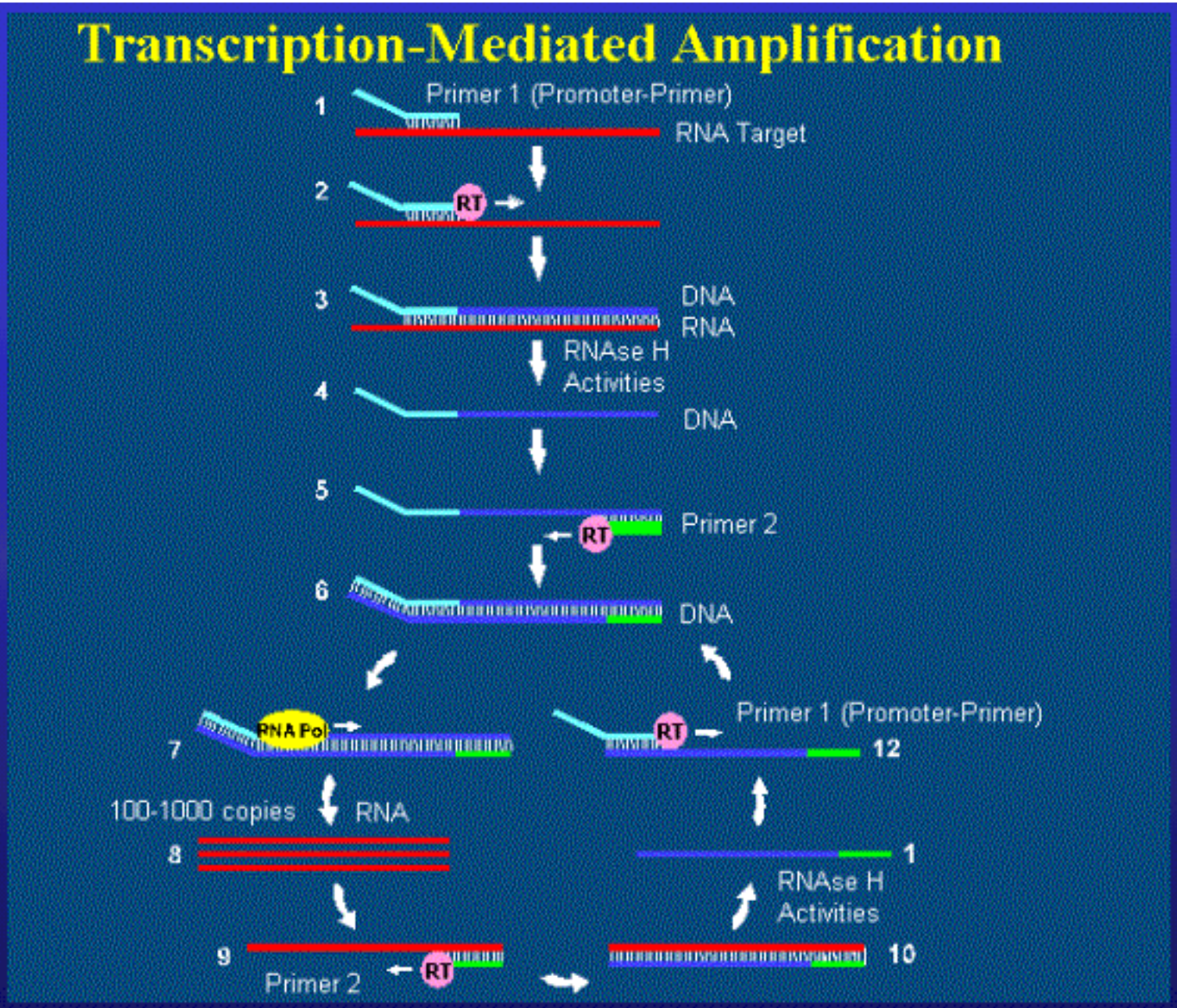
Åbne Spørgsmål???

- Vil HPV vaccines forebygge cervix cancer incidence og mortalitet?
- Er én vaccine series tilstrækkeligt eller vil en "booster" dosis være nødvendig?
- Vil exposition til HPV virke som naturlig boosting?
- Vil nye HPV genotyper overtage HPV16 og 18s plads?
- Hvordan vil vaccinations programmet påvirke cytologiske screenings programmer?
- Er der sjælne men alvorlige bivirkninger ved vaccination?

Gen-Probe Proprietary Target Capture Technology



Transcription-Mediated Amplification



Why Target Capture?

- Reduces false negatives by removing inhibitors
- Simplifies sample processing
- Large sample volumes can be used to accommodate numerous specimen types
- Increases assay specificity

Figure 2. Principle of NASBA

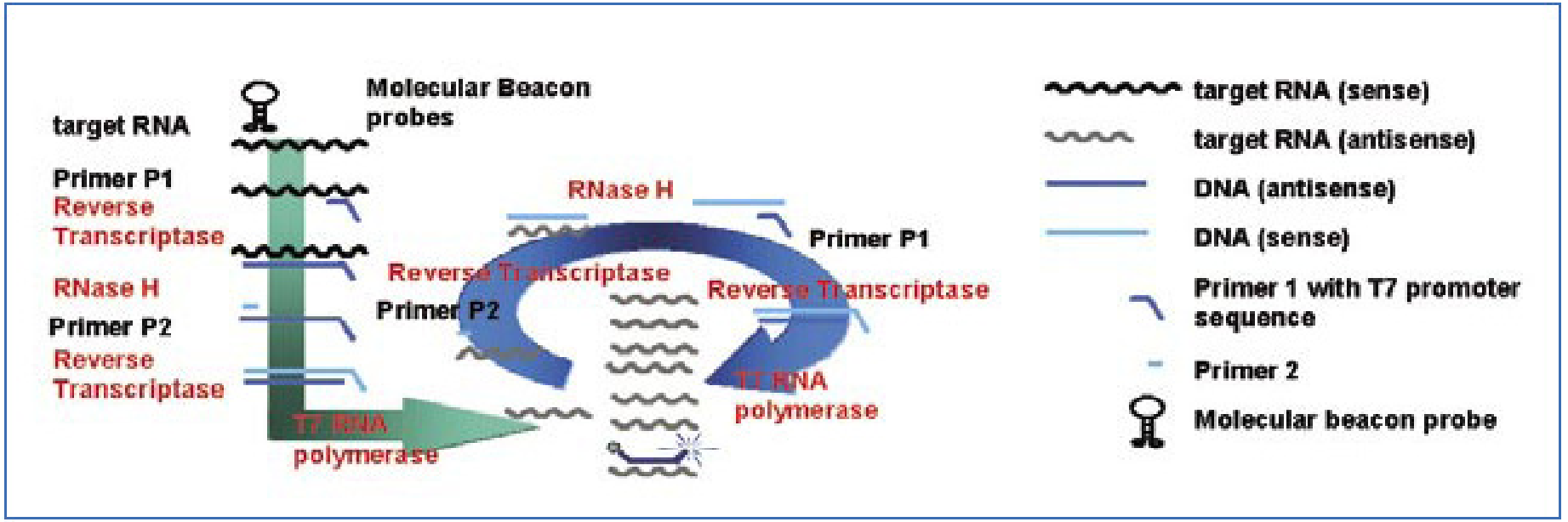
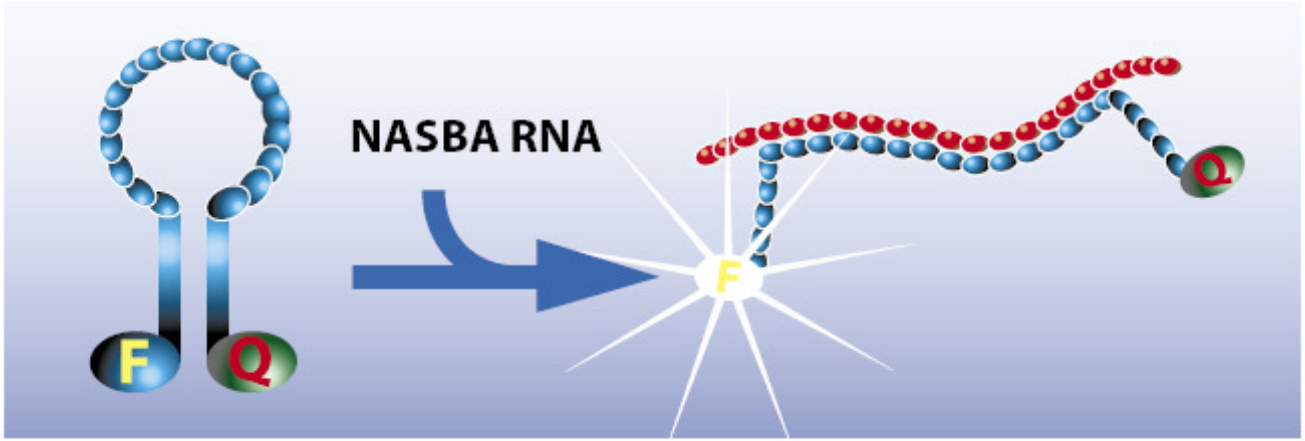
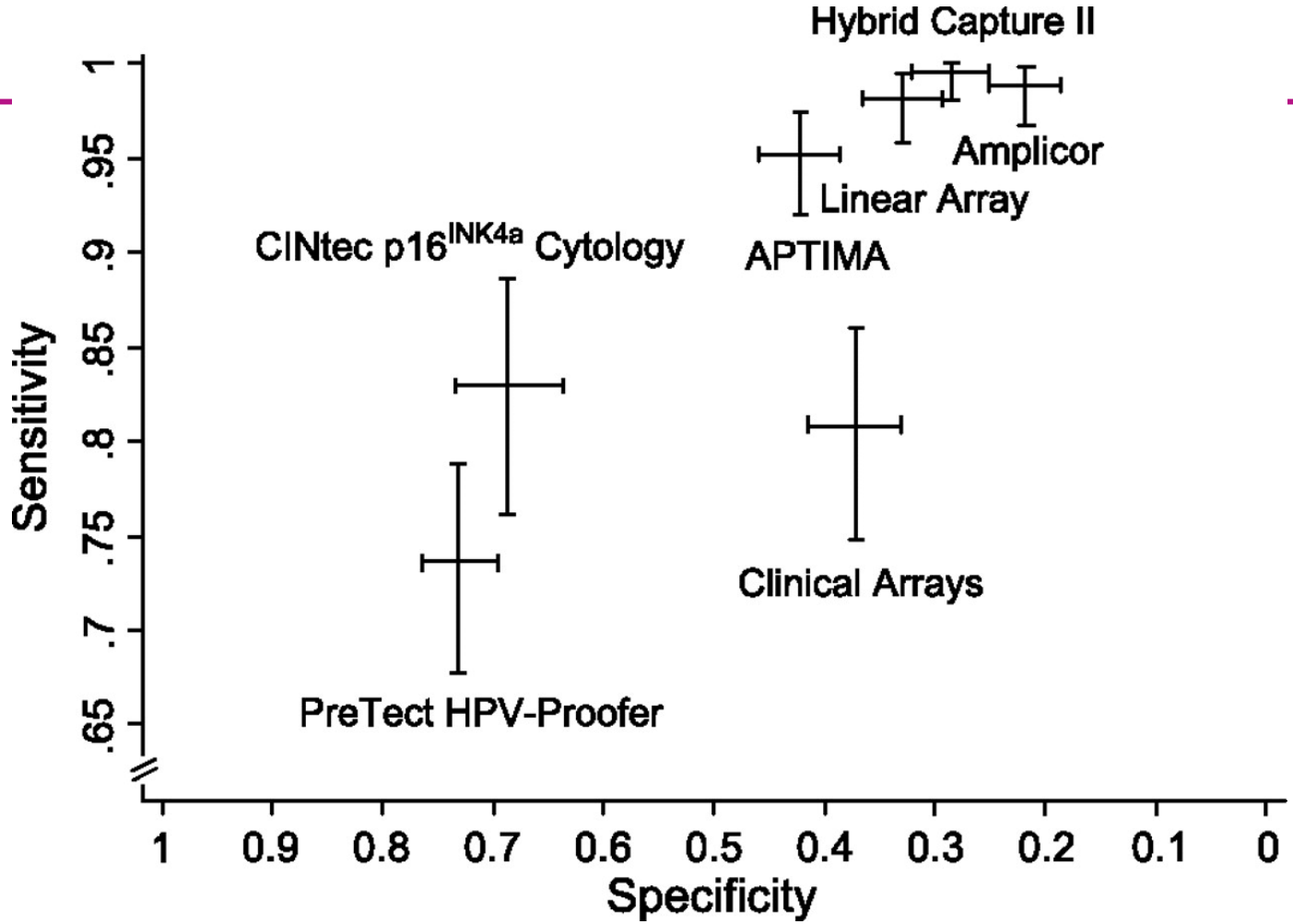


Figure 3. Principle of Molecular Beacon



Molecular Beacons are DNA probes with modified ends. In the folded state (stem-loop) the fluorophore is quenched, but upon binding of the loop sequence to its complementary target sequence the probe undergoes a conformational change and a fluorescence signal is emitted. The probes will hybridize to the anti-sense RNA transcripts that are produced during the transcriptional phase of the NASBA reaction. While amplification proceeds, fluorescent signals are measured real-time in a fluorescent reader.

Figure 4. Summary graph of the sensitivity and specificity results for the detection of CIN2+ (with 95% CI)



Szarewski, A. et al. Cancer Epidemiol Biomarkers Prev 2008;17:3033-3042

Cancer Epidemiology, Biomarkers & Prevention

Test (no. assessed) Ref:A.Szarewski et al	Sensitivity (95% CI) Nov.2008	Specificity (95% CI)	PPV (95% CI)
Hybrid Capture II (*n* = 567)			
CIN2+	100.0 (95.9-100.0)	28.2 (24.2-32.4)	20.4 (16.7-24.5)
Amplicor (*n* = 573)			
CIN2+	97.7 (91.9-99.7)	22.2 (18.6-26.2)	18.4 (14.9-22.2)
PreTect HPV-Proofer (*n* = 558)			
CIN2+	70.6 (59.7-80.0)	74.4 (70.2-78.3)	33.1 (26.3-40.5)
APTIMA (*n* = 573)			
CIN2+	96.6 (90.4-99.3)	42.3 (37.8-46.8)	23.3 (19.0-28.0)
CINtec p16^{INK4a} Cytology (*n* = 323)			
CIN2+	66.7 (52.5-78.9)	68.8 (62.9-74.3)	30.0 (22.0-39.0)
Linear Array (*n* = 562)			
CIN2+	96.6 (90.4-99.3)	34.2 (29.9-38.6)	21.4 (17.5-25.8)
Clinical-Arrays (*n* = 456)			
CIN2+	80.9 (69.5-89.4)	37.9 (33.0-42.9)	18.6 (14.3-23.5)

- Tak for jeres opmærksomhed!

Yin Ling Woo et al jviromet 2007

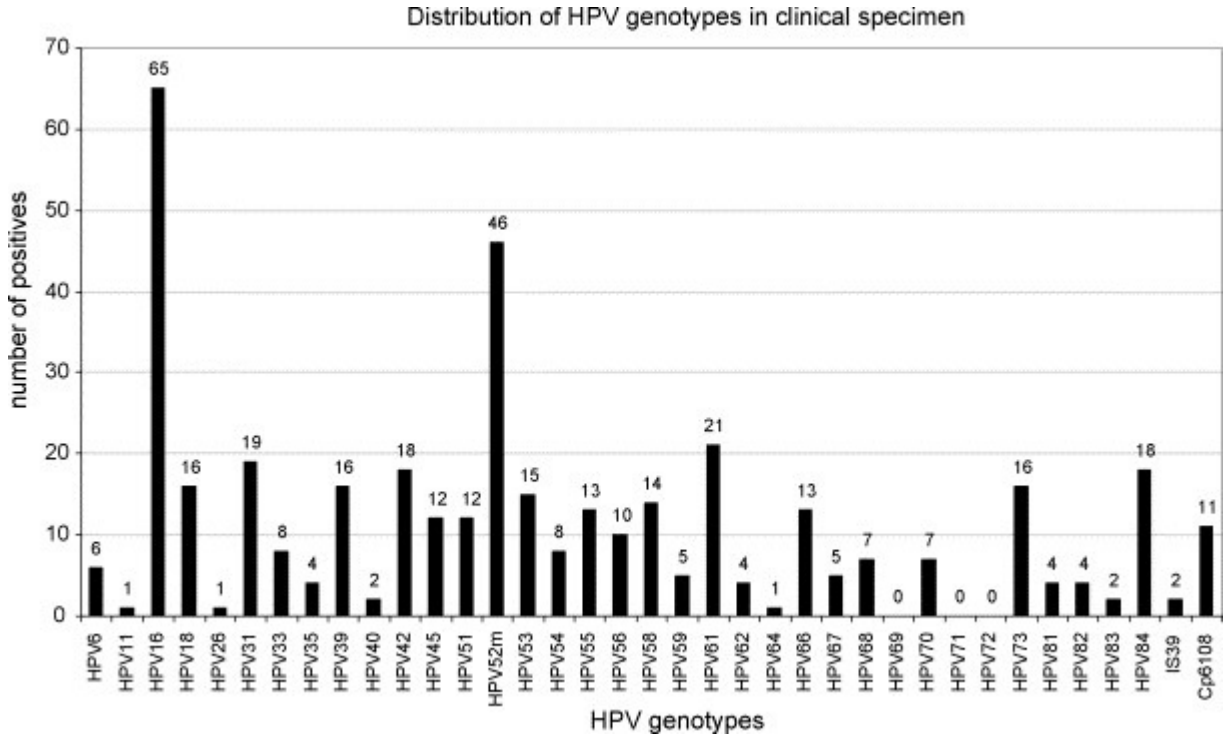


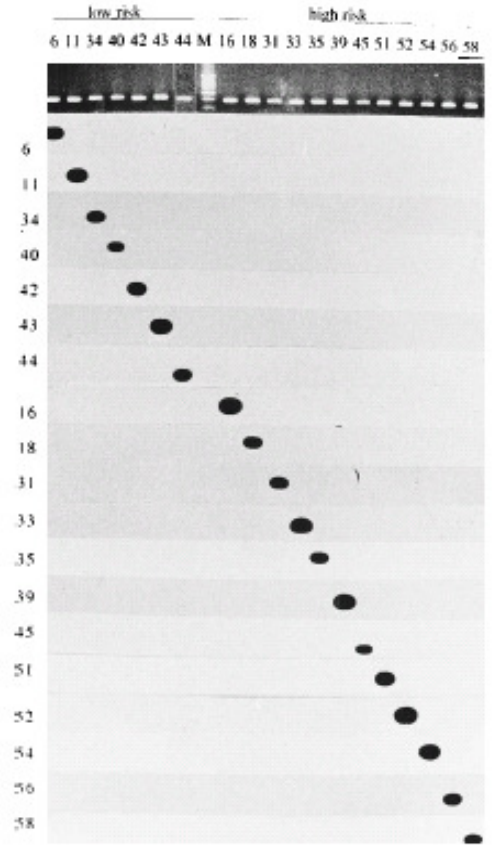
Fig. 3. Distribution of HPV types by Linear Array in all specimens.

2. PCR with General Primers

- HPV: 2,6,11,13,16,18,26,31-35,39,40-45,51
- ,59,61,62,64,66,70,72,73,
  AE2,PAP155,PAP291 and W13B

- Gp5+/gp6+ in L1
- My 09/11 in L1

PCR –gp5+/gp6+ HPV-L1



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3. Hybrid Capture

HPV-16,18,31,33,35,39,45,51,52,56,58,59,68.

Figure 3

Basic steps of the Hybrid Capture assay

1. Release nucleic acids
Clinical specimens are combined with a base solution which disrupts the virus or bacteria and releases target DNA. No special specimen preparation is necessary.

2. Hybridize RNA probe with target DNA
Target DNA combines with specific RNA probes creating RNA:DNA hybrids.

3. Capture hybrids
Multiple RNA:DNA hybrids are captured onto a solid phase coated with universal capture anbtibodies specific for RNA:DNA hybrids.

4. Label for detection
Captured RNA:DNA hybrids are detected with multiple antibodies conjugated to alkaline phosphatase. Resulting signal can be amplified to at least 3000-fold.

5. Detect, read and interpret results
The bound alkaline phosphatase is detected with a chemiluminescent dioxetane substrate. Upon cleavage by alkaline phosphatase, the substrate produces light that is measured on a luminometer in Relative Light Units (RLUs).

Courtesy of Digene Corp., used with permission.

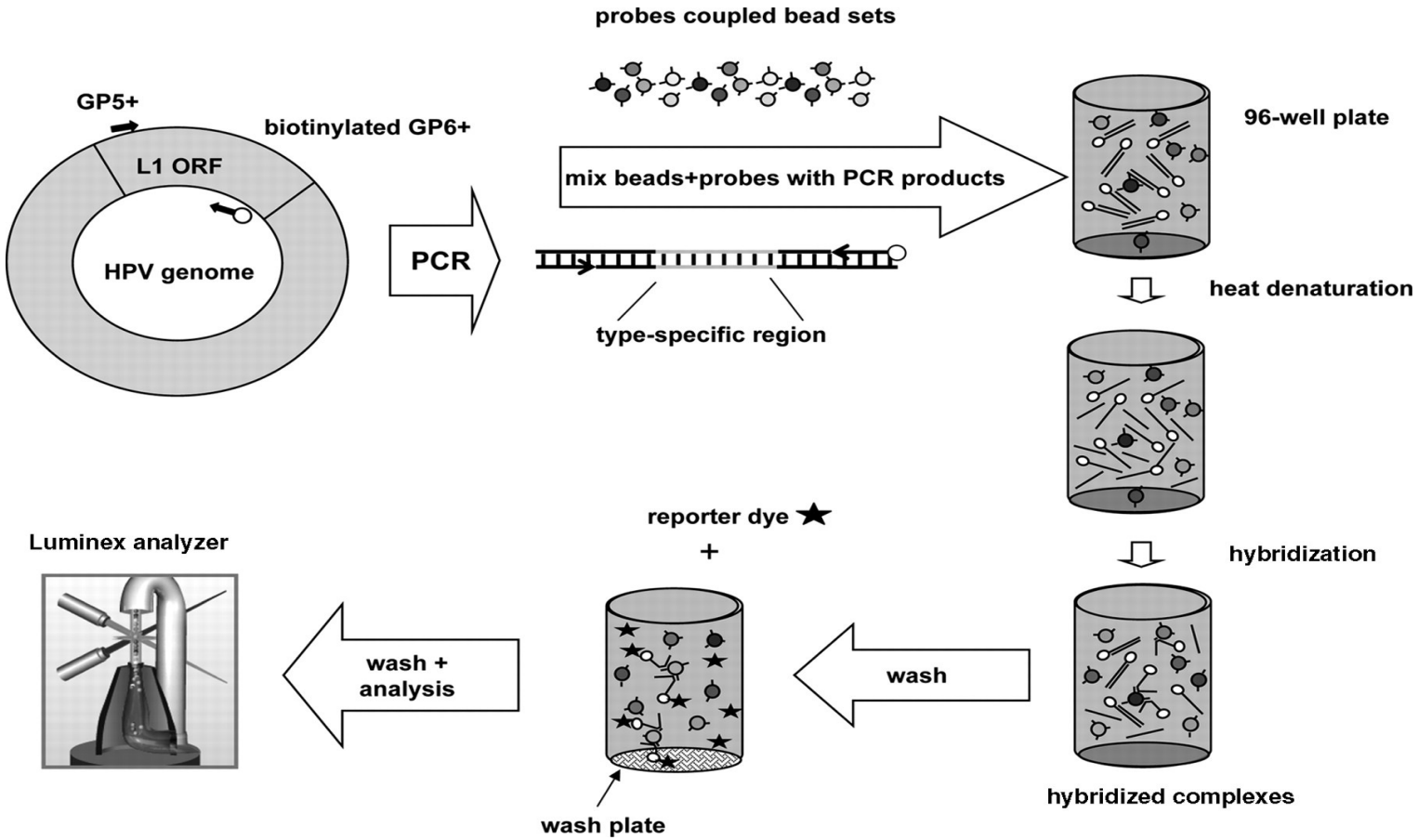
4. LiPA

- Biotinylated primers in L1
- 65 bp fragments
- Hybridization to filters
- 25 different types in the assay

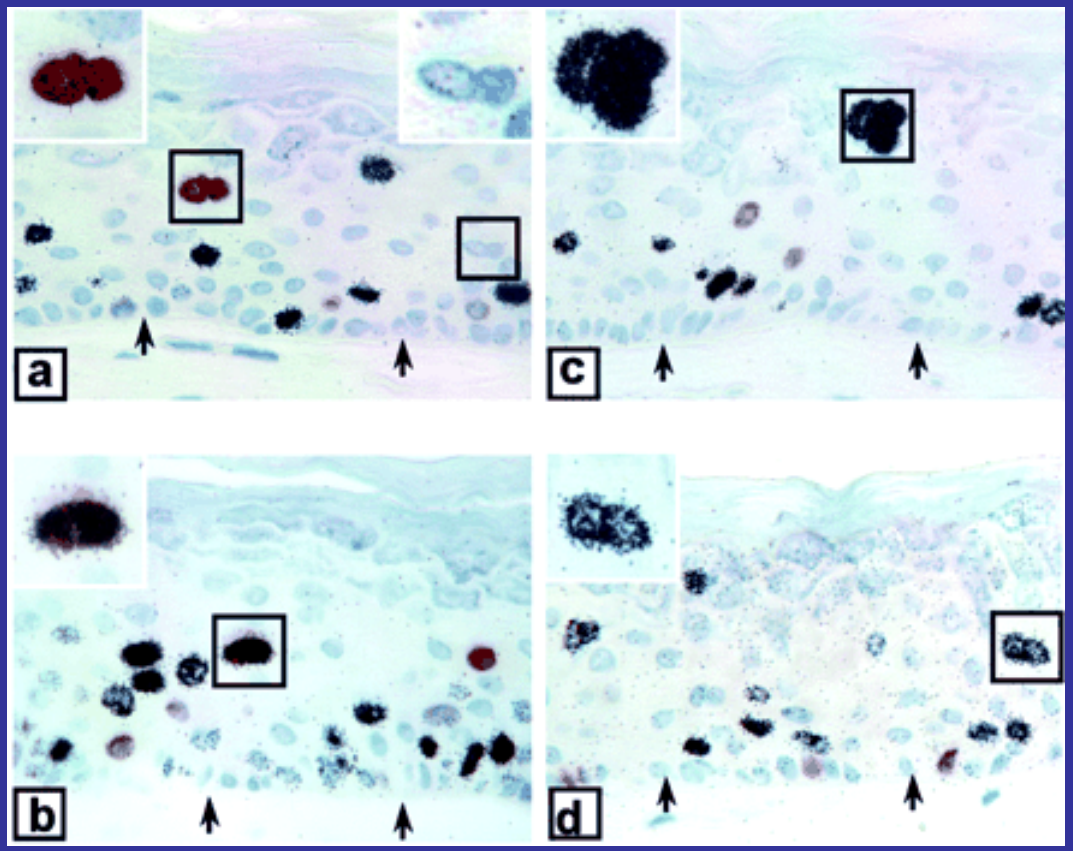
5. PCR with Luminex amplification

Luminex Coorp

Ref. Markus Schmitt et al 2006



6. HPV in Epithelium by in situ hybridization



Chien et al 2002

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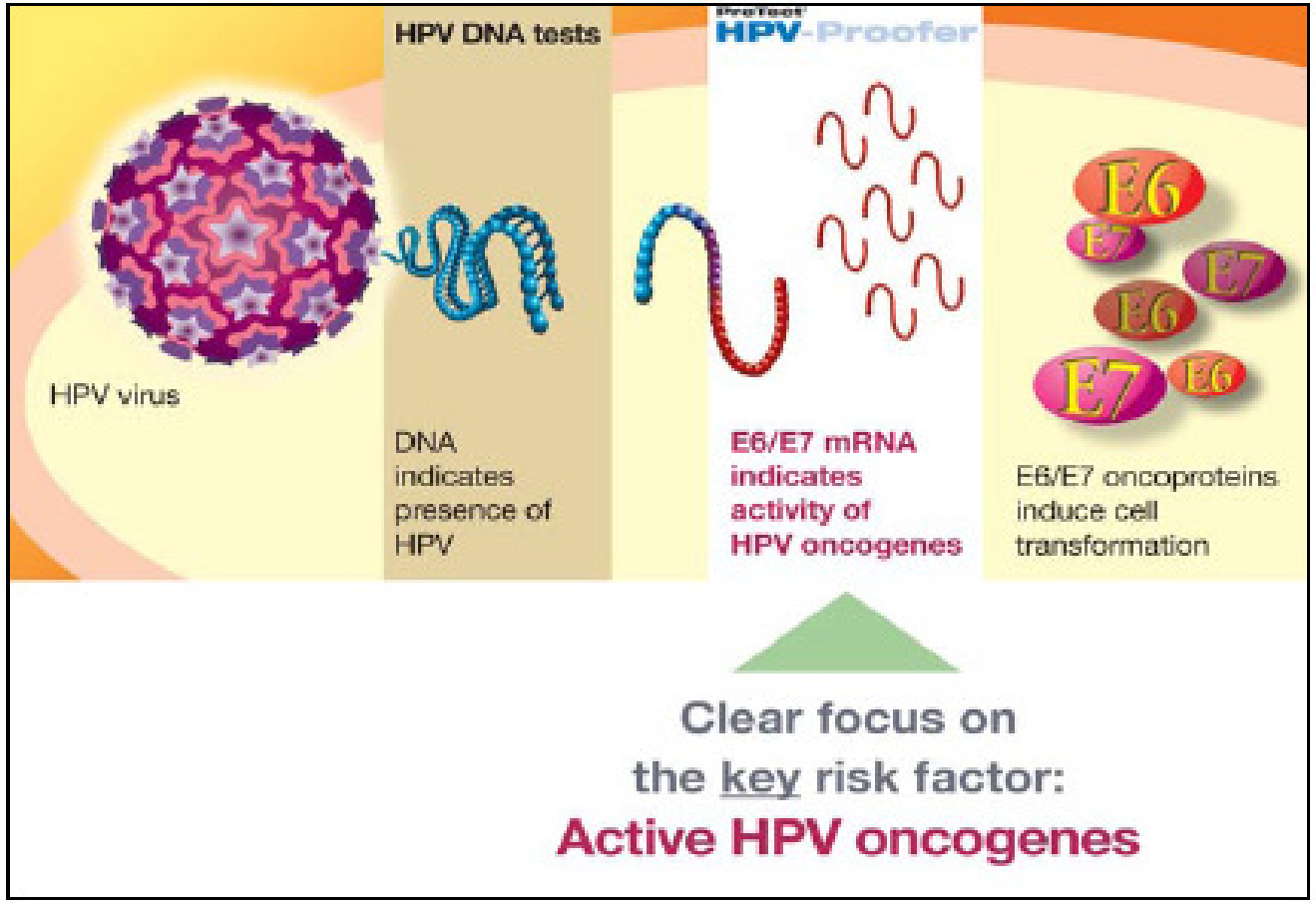
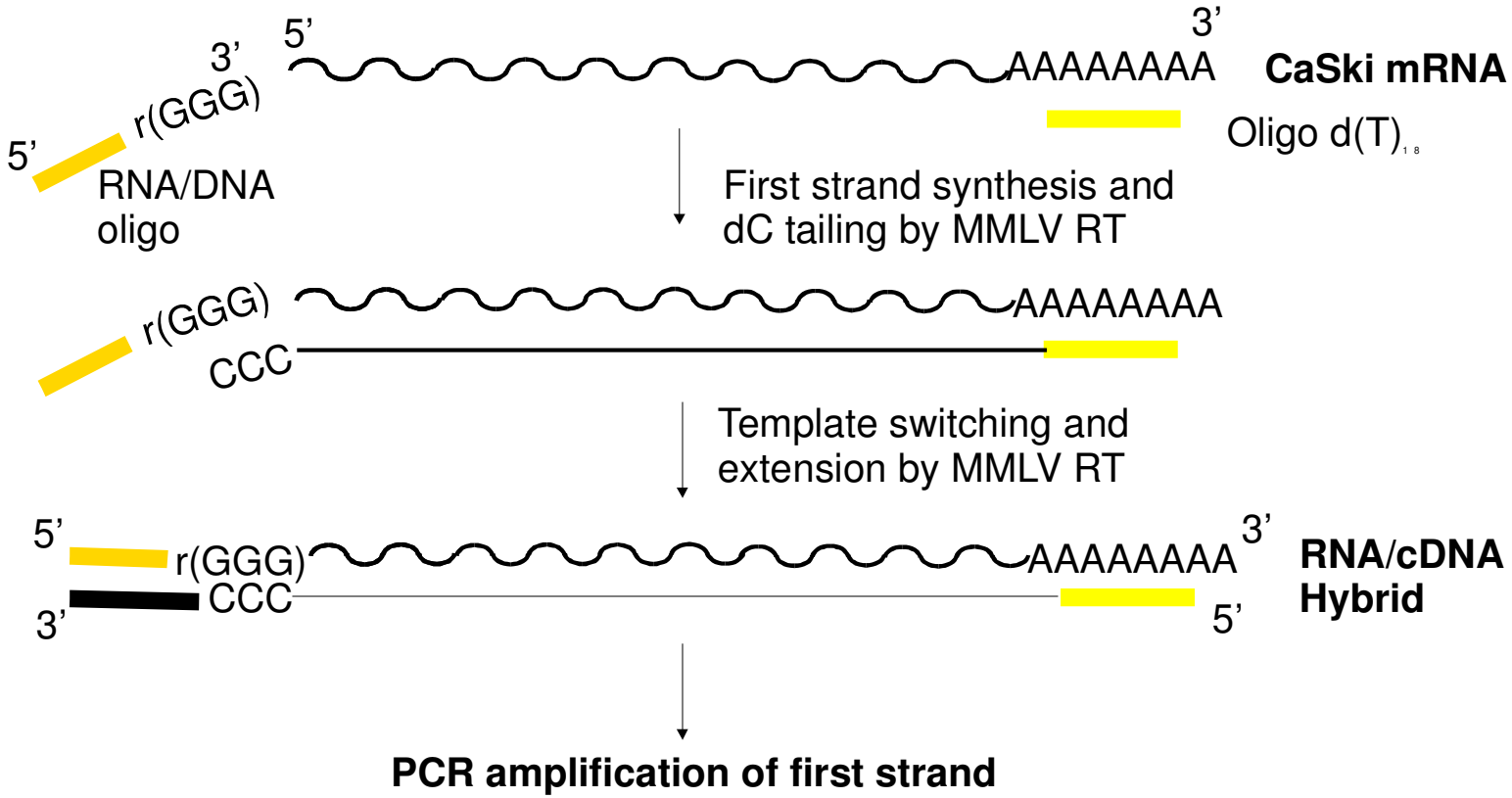
7. HPV mRNA detection

21st. International Papillomavirus Conference, 20-26 February, Mexico City, 2004

Fig 1: HPV-Proofer detect E6/E7 mRNA from the five carcinogenic HPV types indicating activity of HPV oncogenes.



RT-PCR


5'
3'
AAAAAAAA
CaSki mRNA
3'
r(GGG)
5'
RNA/DNA
oligo
Oligo d(T)18
First strand synthesis and
dC tailing by MMLV RT
r(GGG)
CCC
AAAAAAAA
Template switching and
extension by MMLV RT
5'
r(GGG)
CCC
3'
AAAAAAAA
3'
5'
RNA/cDNA
Hybrid
PCR amplification of first strand

NorChip

NASBA

PreTect HPV-Proofer

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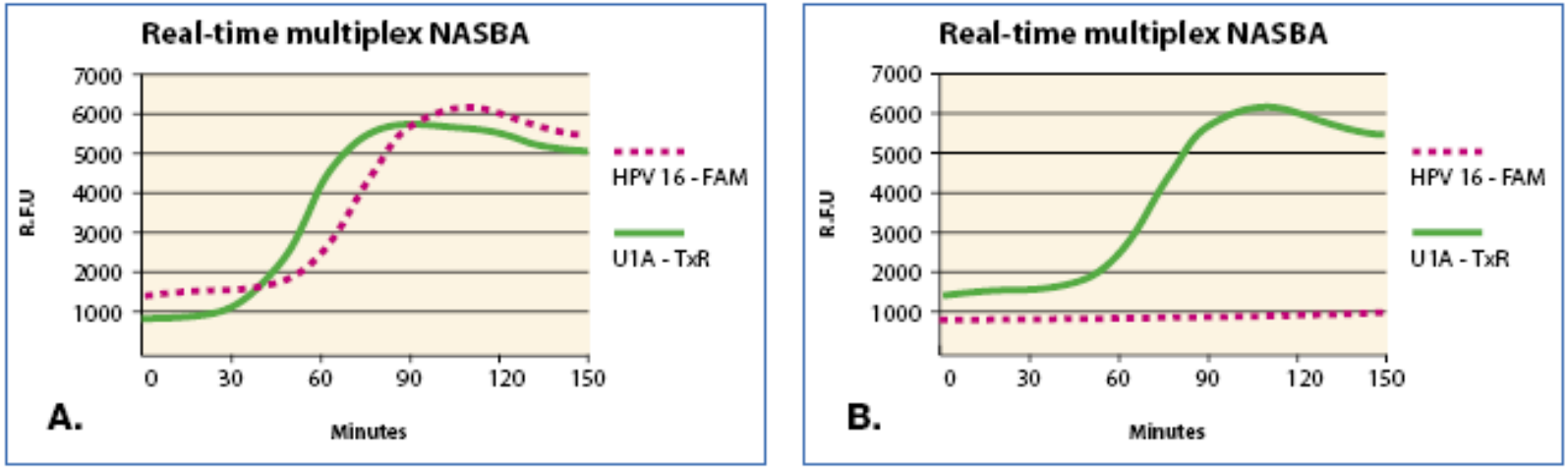
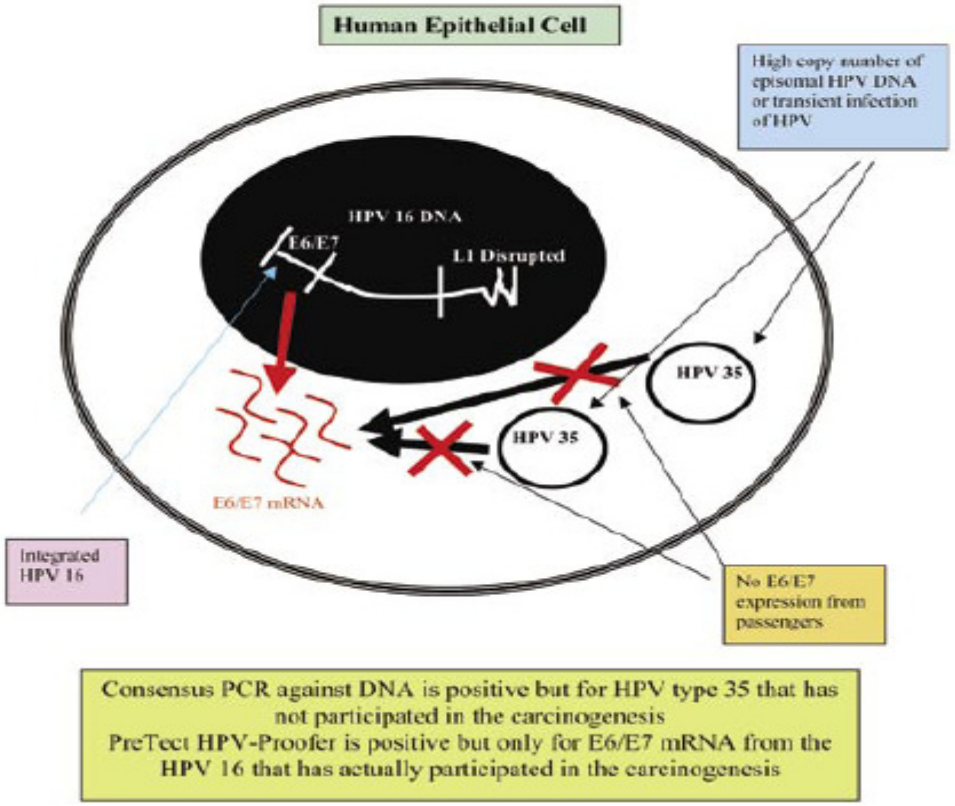
Figure 4. Real-time multiplex Nucleic Acid Sequence Based Amplification (NASBA)



A. Sample positive for HPV 16 mRNA and for the human U1A mRNA internal sample control.
B. Sample negative for HPV 16 mRNA but positive for the human U1A mRNA internal sample control.

Figure 2. Presence of possible HPV "passengers" or non-transforming infections.



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HPV-Proofer correlated with other methods

The HPV-Proofer method was developed to perform typing of HPV types 16, 18, 31, 33 and 45 with concurrent detection of expression markers (bicistronic mRNA of known oncogenes E6 and E7) in order to detect loss of viral and cell cycles regulation presented as a major cell nuclei abnormality. HPV-Proofer was evaluated against the following methods in the below listed clinical studies.

- Type-specific PCR
- Quantitative real-time PCR
- Consensus PCR
- HCII
- In situ hybridization
- Line-blot PCR
- NASBA with end-point ECL detection
- Singleplex real-time NASBA
- Sequencing analysis

21st. International Papillomavirus Conference, 20-26 February, Mexico City, 2004

Table 1: mRNA and DNA analysis in 4136 cervical smears collected in a screening population in Norway.

	Normal	ASCUS	Condyloma	CIN I	CIN II	CIN III	SCC	Total
HPV mRNA	95 2%	12 21%	6 32%	0 0%	2 40%	9 75%	1 100%	126 3%
HPV DNA	368 9%	27 47%	14 74%	1 100%	2 40%	10 83%	1 100%	429 10%
Cytology	3970	57	19	1	5	12	1	4136*

*Results from groups unsatisfactory (64), AGUS (5) and ASC-H (2) not shown

	HC-II	PCR	LiPA	RT-PCR
Positive	•Reproducible •Commercial •Large screen •Easy	•Sensitive •Small sample •Large screen •HPV typing	•Sensitive •Small sample volume •Large screen •HPV typing •Commercial	•Specific for expression •Commercial •Large screen •Quantitative
Negative	•Low sensitivity •Sample vol > •Cross reactivity	•Less reproducible •Contamination •Not commercial	•Low risk HPV •Less reproducible •Cross reactivity	•Less Types •Sample vol> •High Quality of samples

Figure 1. APOT - Amplification of Papillomavirus Oncogene Transcripts

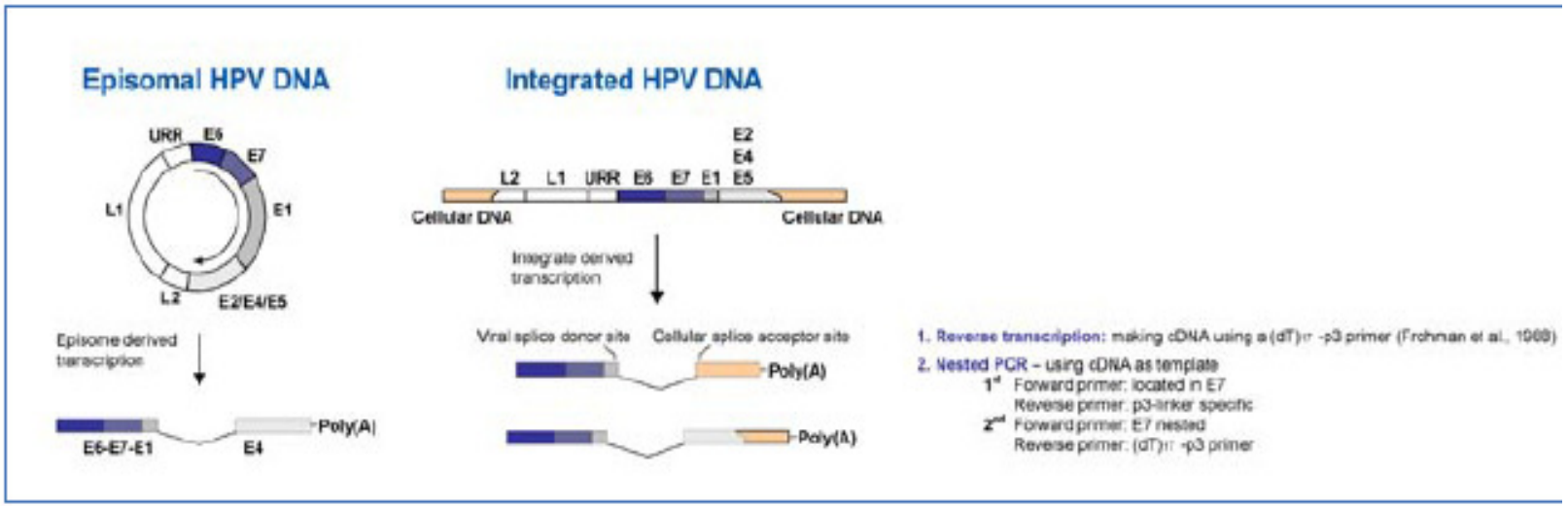
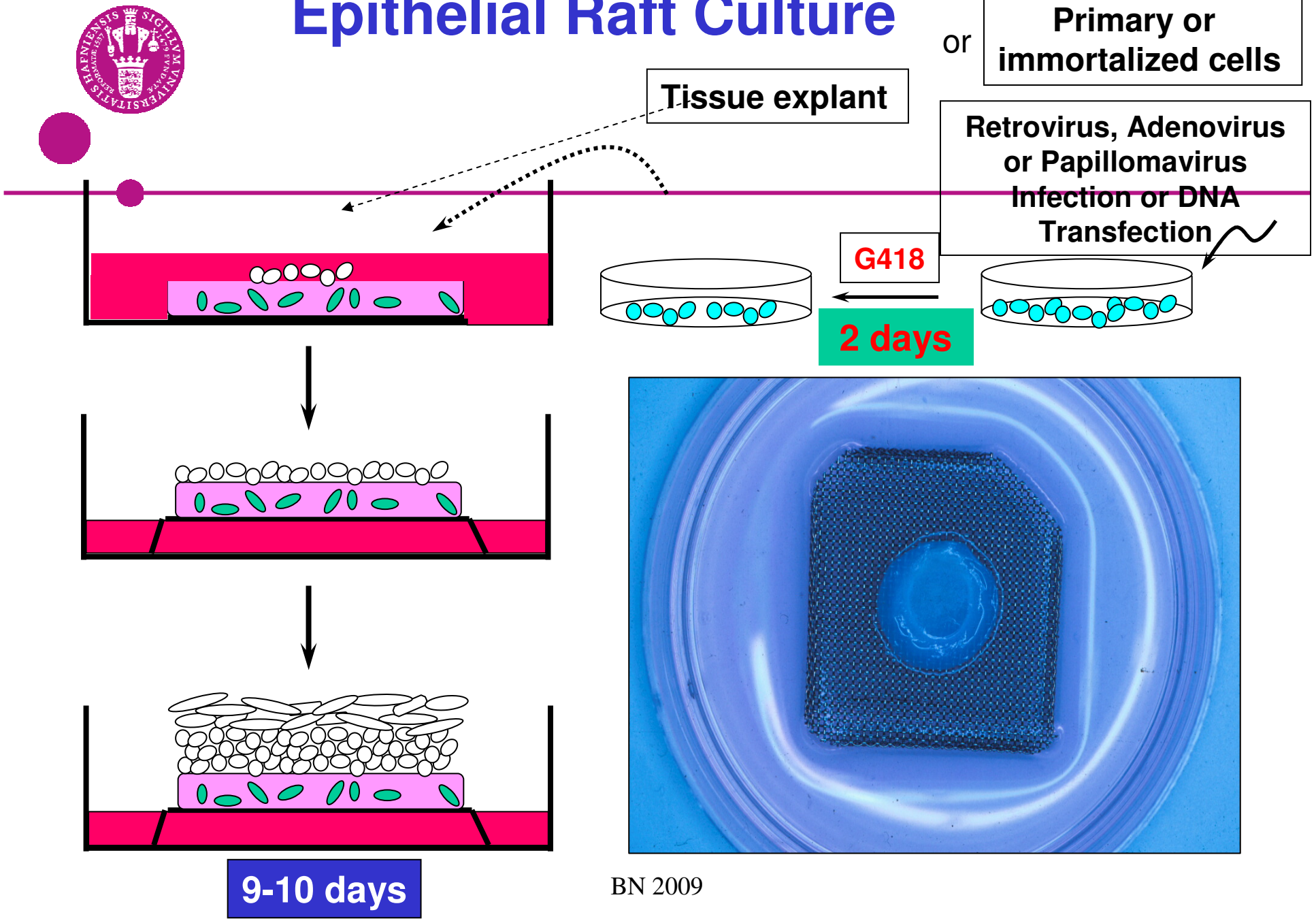


Table 1. Frequency of viral-cellular fusion transcripts

	HPV 16 (Frequency %)	HPV 18 (Frequency %)
Episomal	16 (48,5%)	5 (21,7%)
Episomal+ Integrated	4 (12,1%)	1 (4,3%)
Integrated	13 (39,4%)	17 (74%)
Total	33 (100%)	23 (100%)

Discussion

Epithelial Raft Culture



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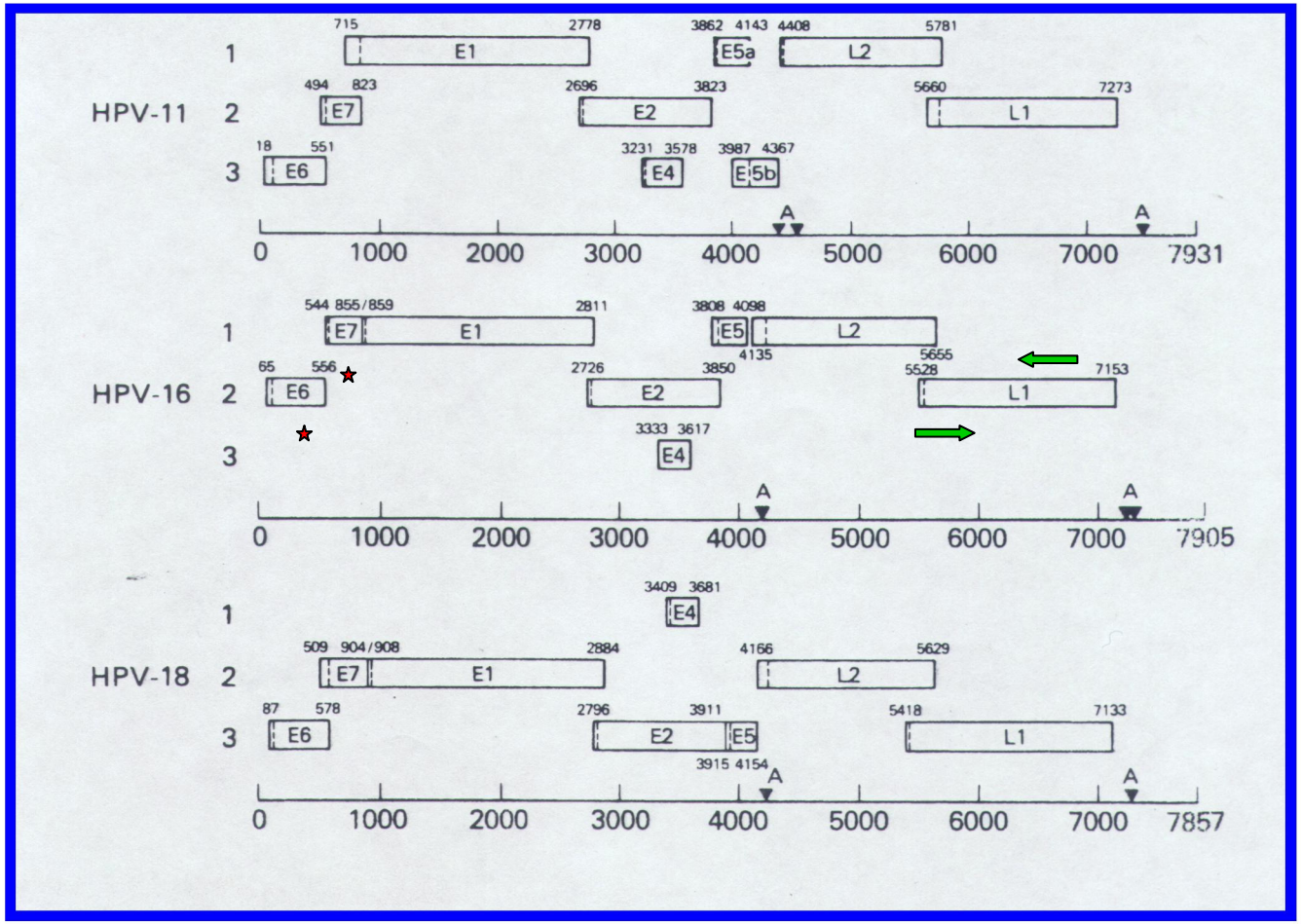
HPV Genomes



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