

Human Papillomavirus (HPV) *Diagnosis*

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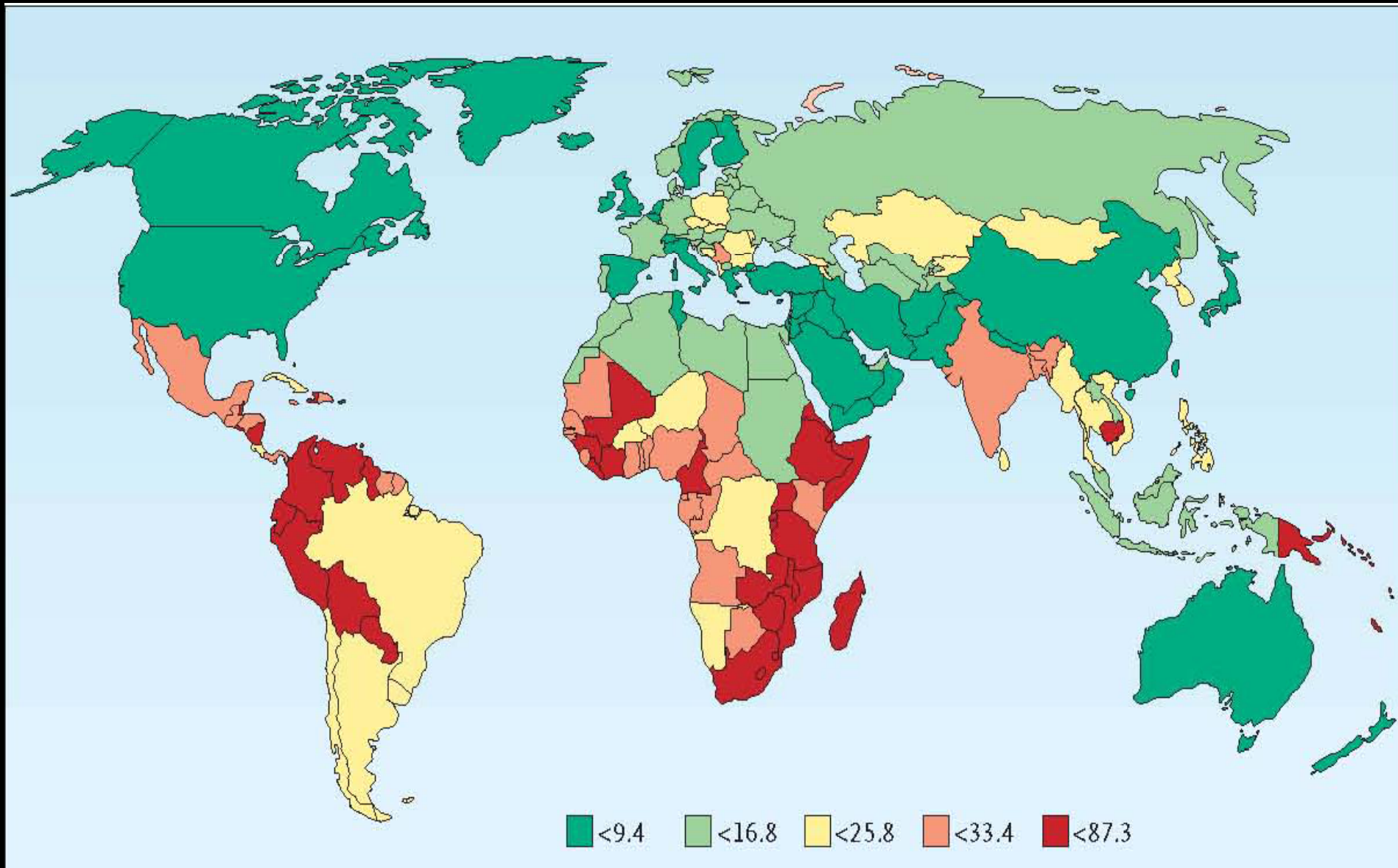


WHO Regional Reference Laboratory
for HPV Diagnosis and Control



Cervical Cancer

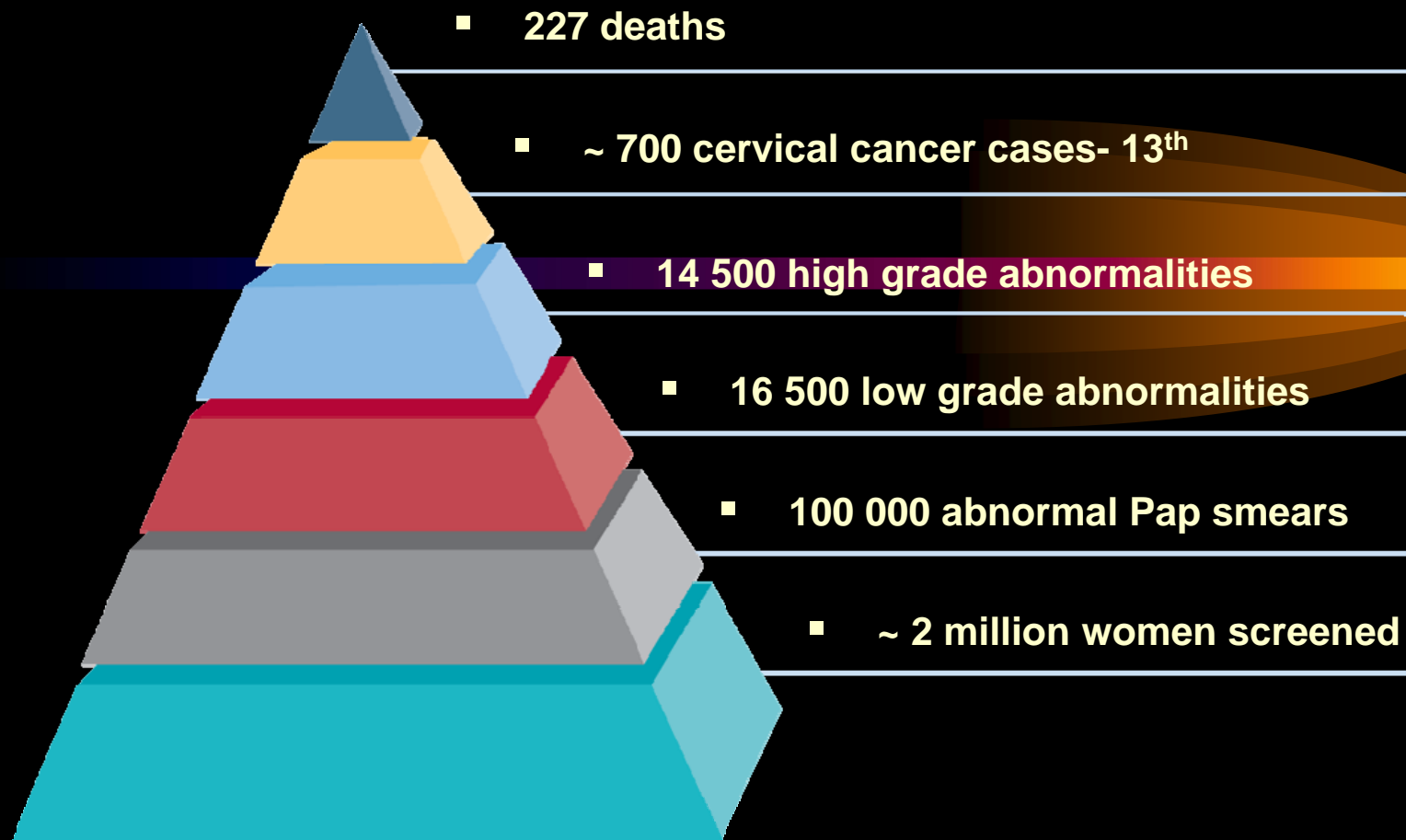
- 2nd most common cancer of women worldwide
 - ~ 1/2 million new cases annually (80% in developing countries)
 - Cancer usually appears in people aged >35



Incidence of Cervical Cancer Worldwide.

Numbers indicate cases per 100,000 population.

Burden of cervical disease in Australia

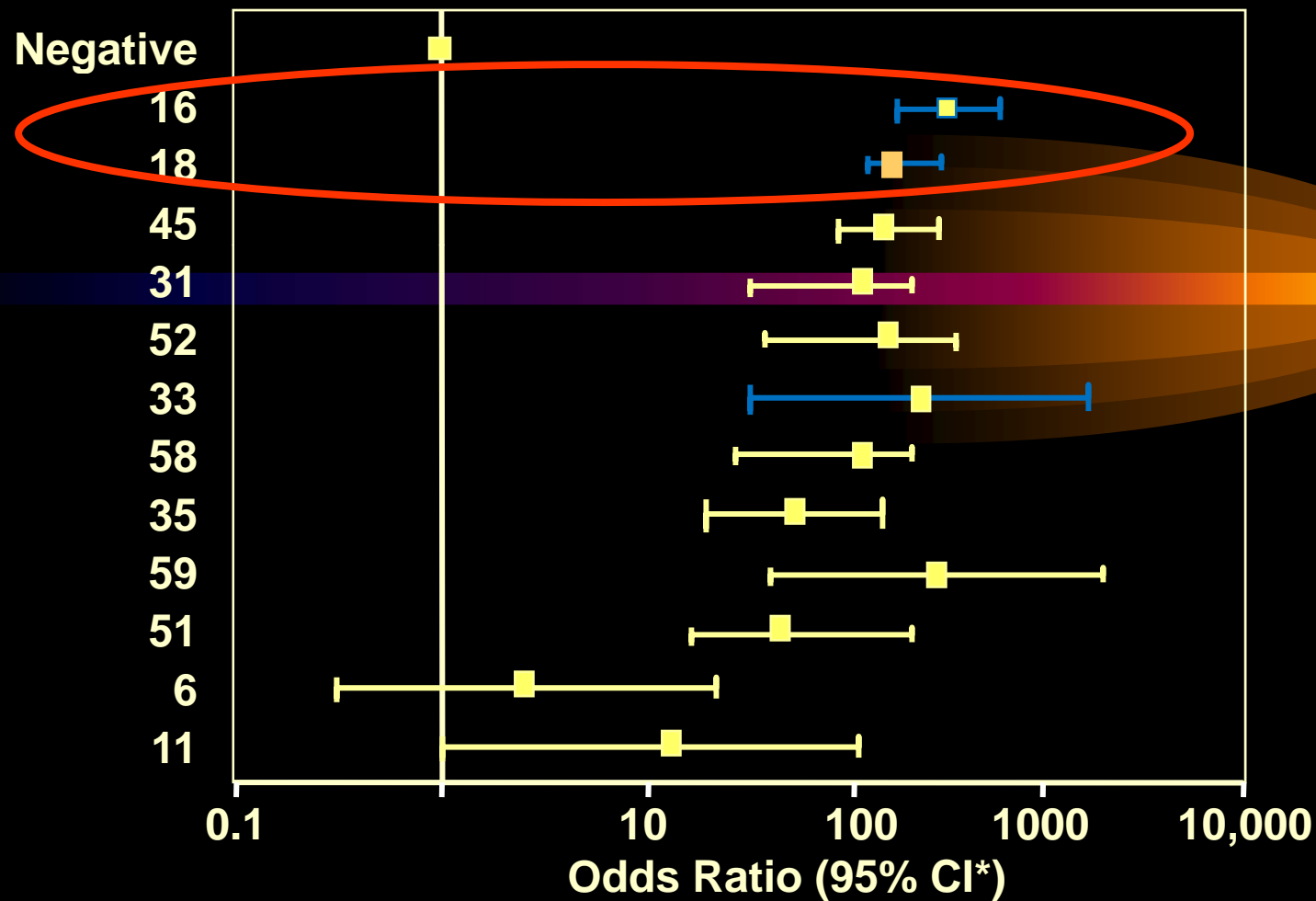


* Annual events. 1. NHMRC. 2005. 2. AIHW, 2006. 3. Blomfield *et al*, 2005

Cervical Cancer and Human papillomavirus (HPV)

- ~ 99% of ICC cases have detectable HPV DNA
- Molecular & *in vitro*
- epidemiological association
- HPV 16 and 18 are recognized carcinogen

Risk of Cervical Cancer by HPV Type



*CI = confidence interval

Muñoz N, Bosch FX, de Sanjosé S, et al. *N Engl J Med.* 2003;348:518–527.

Cancer	Relative Risk
Lung Cancer- smoking	10
Liver Cancer- HCV	20
Liver Cancer- HBV	50-100
Cervical Cancer- HPV	300-500

Other HPV infection

- Vulvar Cancer (50%)
- Recurrent Respiratory Papillomatosis [RRP] (100%)
- Head & neck cancers (28%)
- Anal cancer (85%)
- Penile cancer (80%)

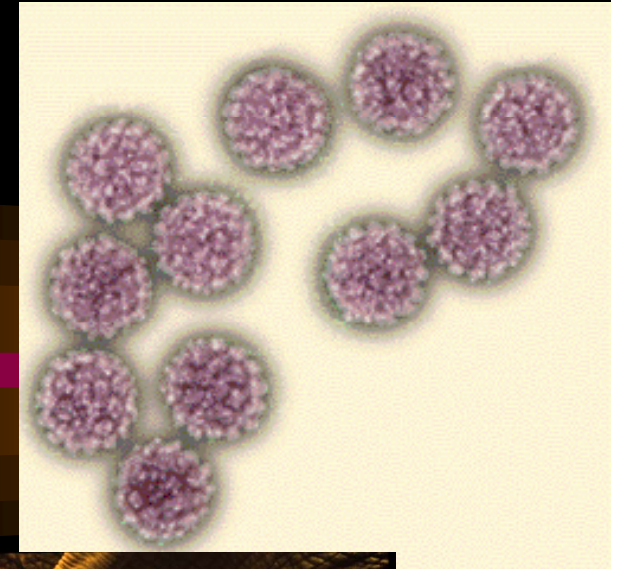
Professor Harald zur Hausen

Joint Nobel Prize winner
in Physiology /Medicine 2008



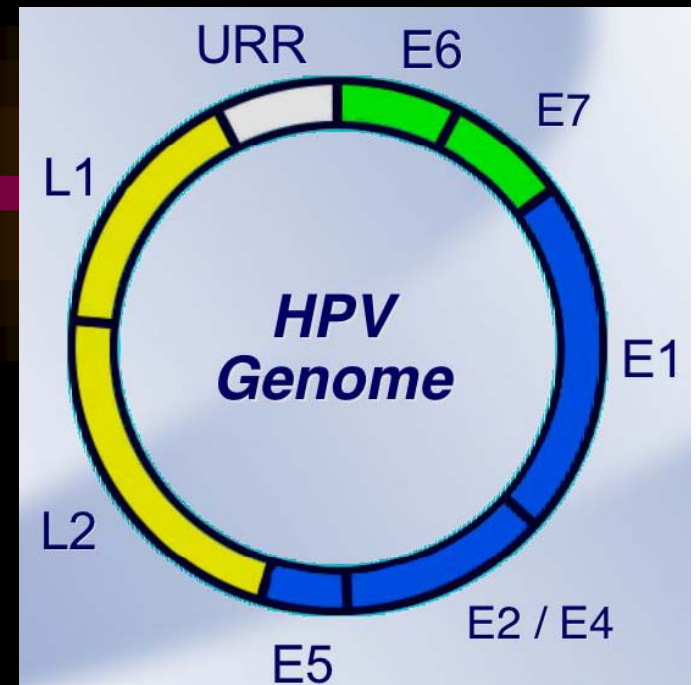
Papillomaviruses

- Family: Papillomavirus
- Non-enveloped dsDNA viruses
 - 55-nm spherical capsid coat
- Tropism for squamous epithelium
- Widely distributed in higher vertebrates
 - Tight species specificity



Human papillomavirus

- DS ~8kb , static (recombination and mutations are rare events)
- Early region
 - Six ORF-
 - E6 and E7 transforming oncoprotein
 - Rest are required for viral replication
- Late region
 - Encodes for 2 viral capsid proteins
 - L1 ORF - most conserved and used for identification of new PV types
- Upstream regulatory region
 - Sequences that control transcription of the viral genome



Human Papillomavirus

- Cannot be routinely propagated in-vitro
- Detection and typing is based on nucleic acid sequence
 - >10% sequence variation = new type;
2-10% = subtype, <2% = variant
- Types assigned sequential number based on order of discovery
 - No relation to phylogeny
- Heterogeneous group of ~ 200 genotypes



- Two major branches, differing affinities for site of infection
- **Cutaneous:** Keratinized squamous epithelium
- **Mucosal:** Non-keratinized squamous epithelium

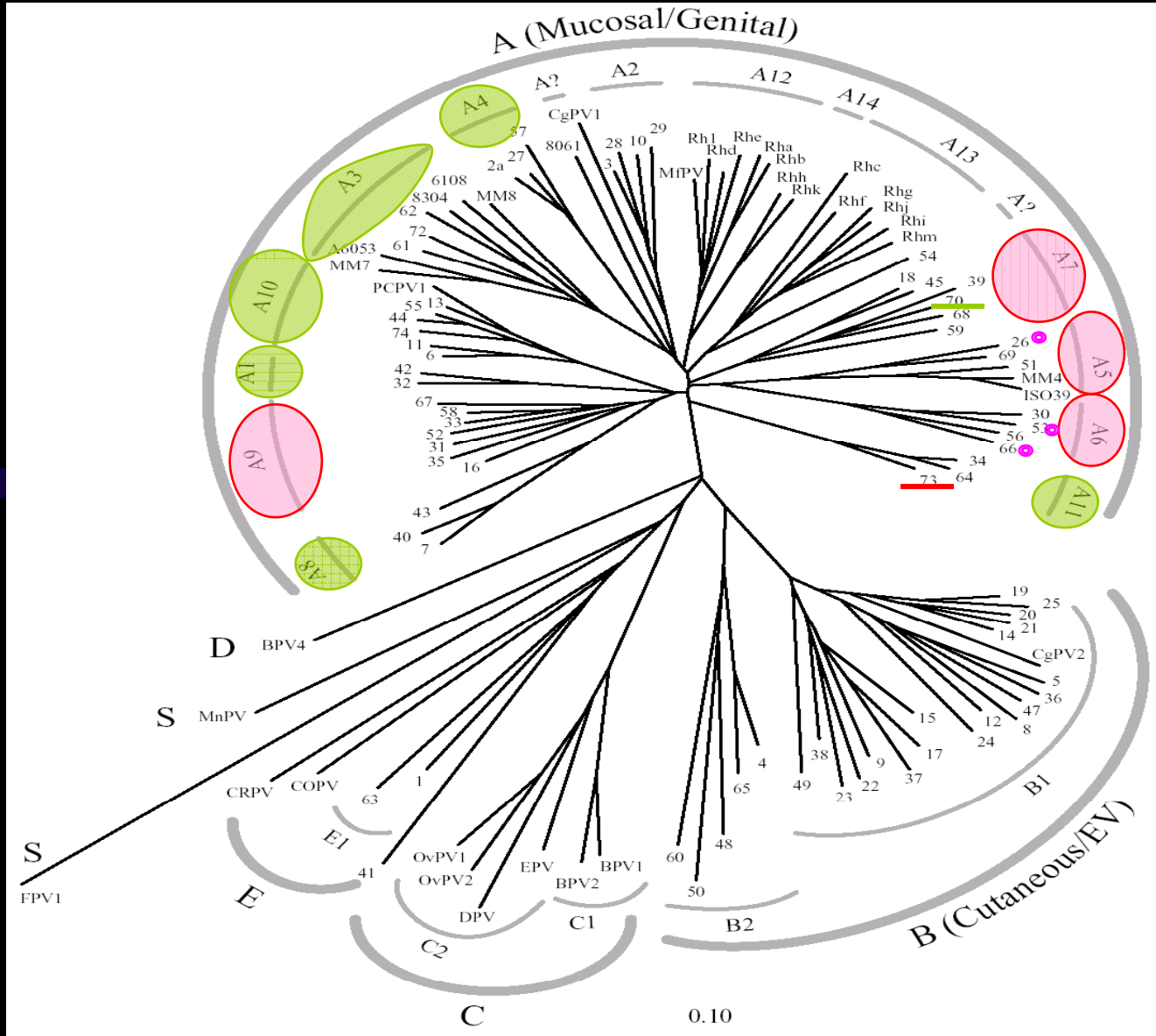
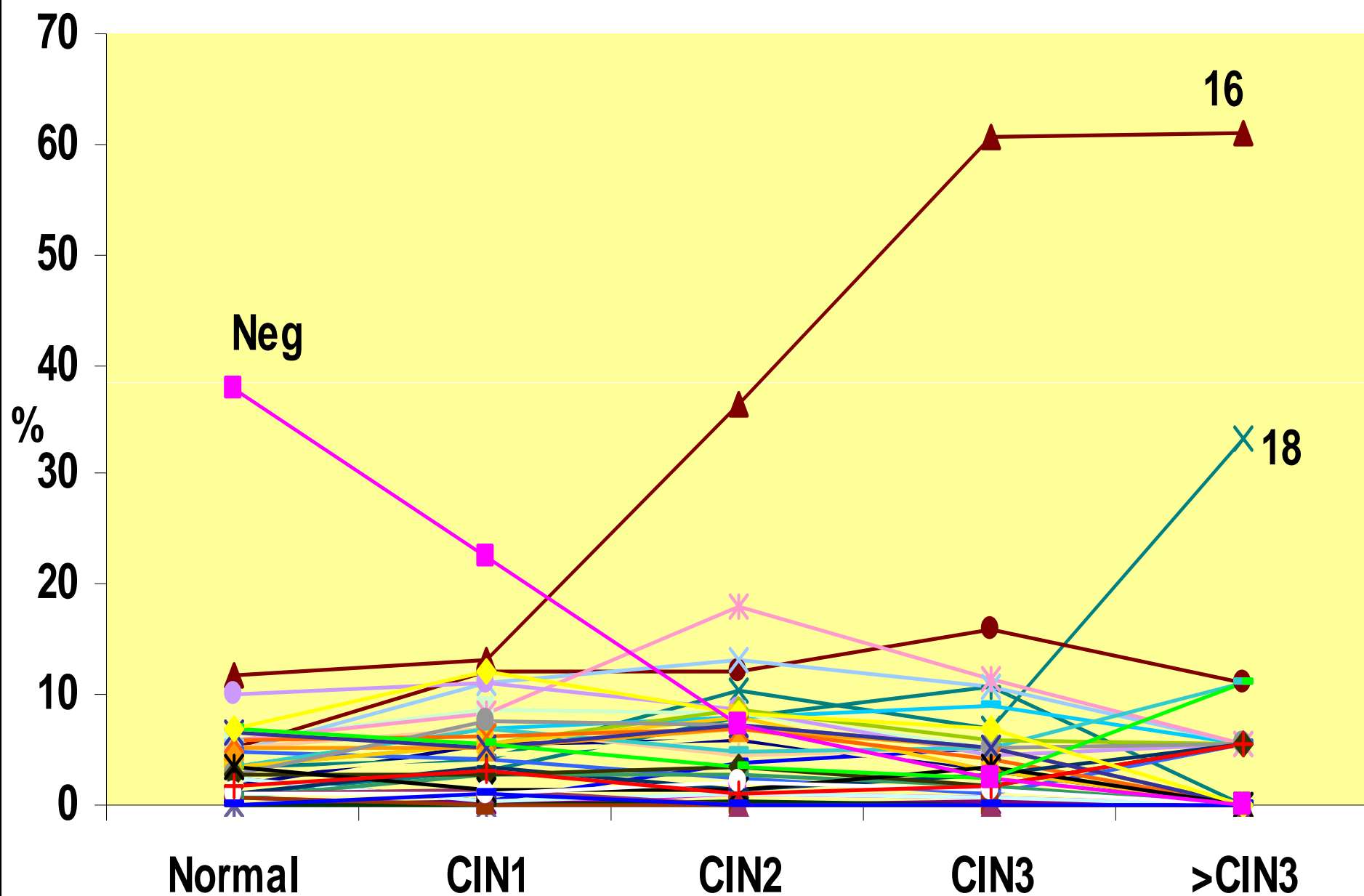
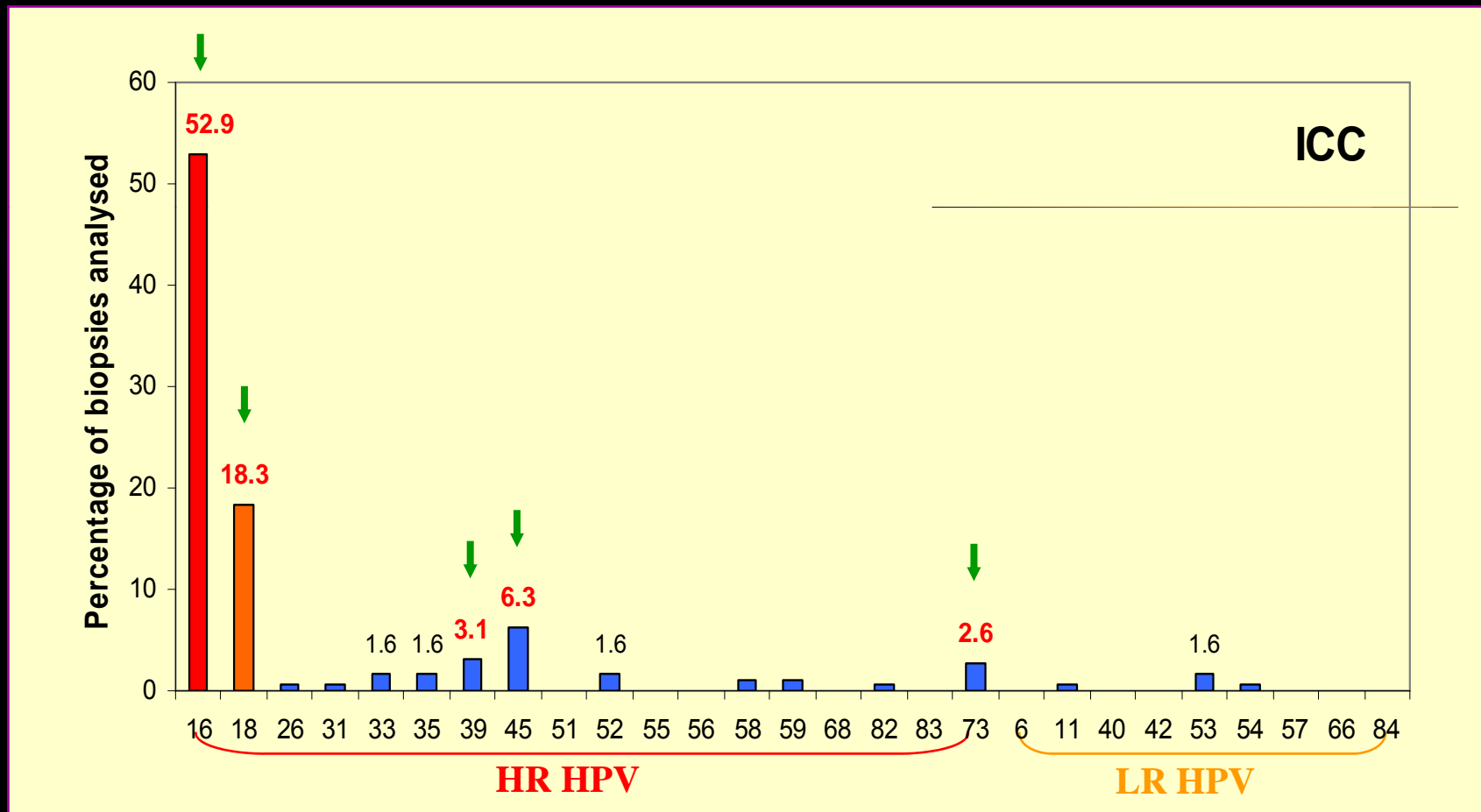


Figure 1. Neighbor joining phylogenetic tree of 106 PVs based on CPR region of L1.



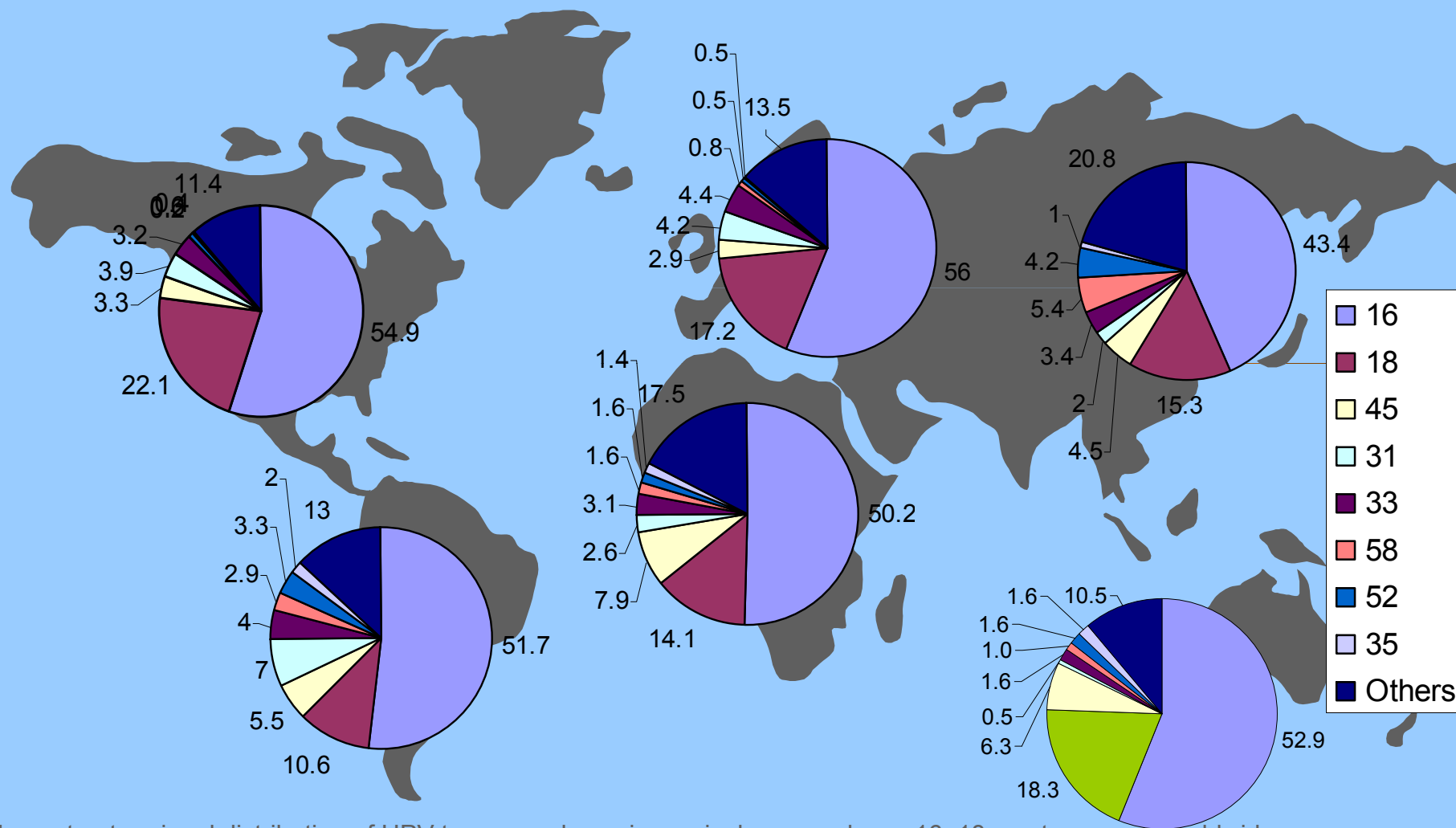
HPV prevalence in ICC biopsies

(n = 191)



Predominant types: 16, 18, 45, 39 and 73

Geographical variations: cervical cancer

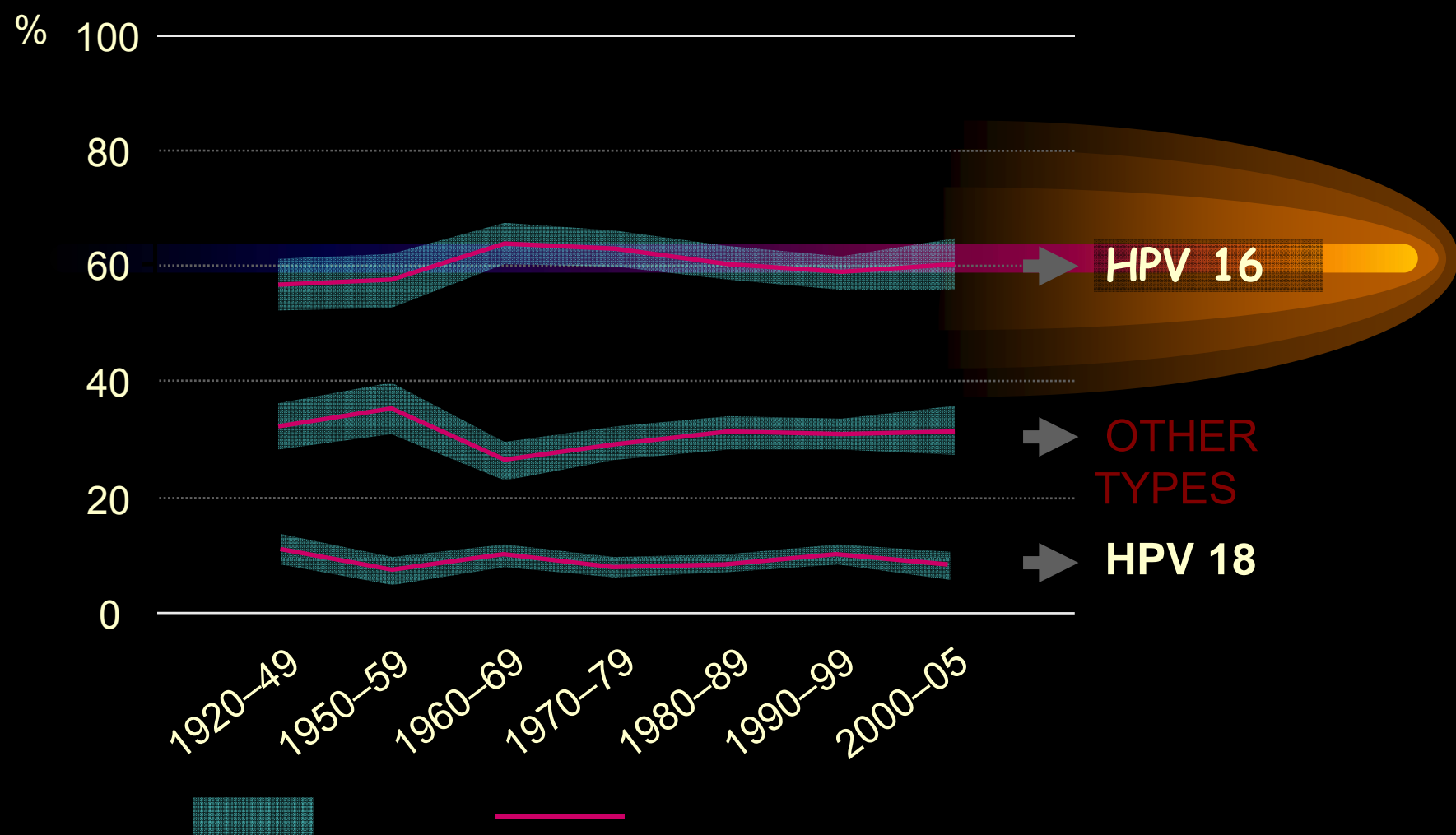


In contrast regional distribution of HPV type prevalence in cervical cancer shows 16, 18 most common worldwide; thereafter geographical variation.

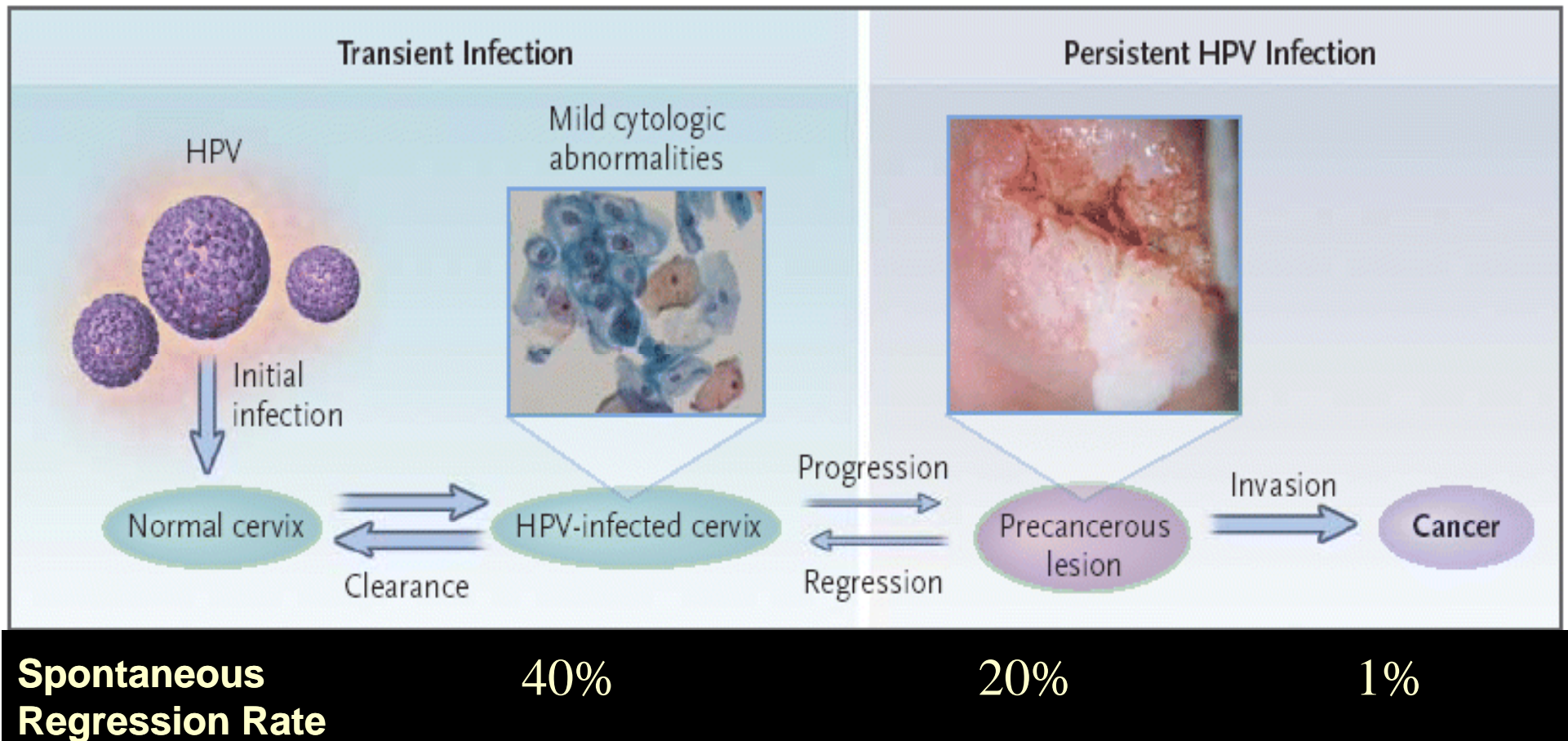
Clifford GM, et al., (2003) HPV types in invasive cervical cancer worldwide: a meta-analysis. Br J Cancer 88: 63–73

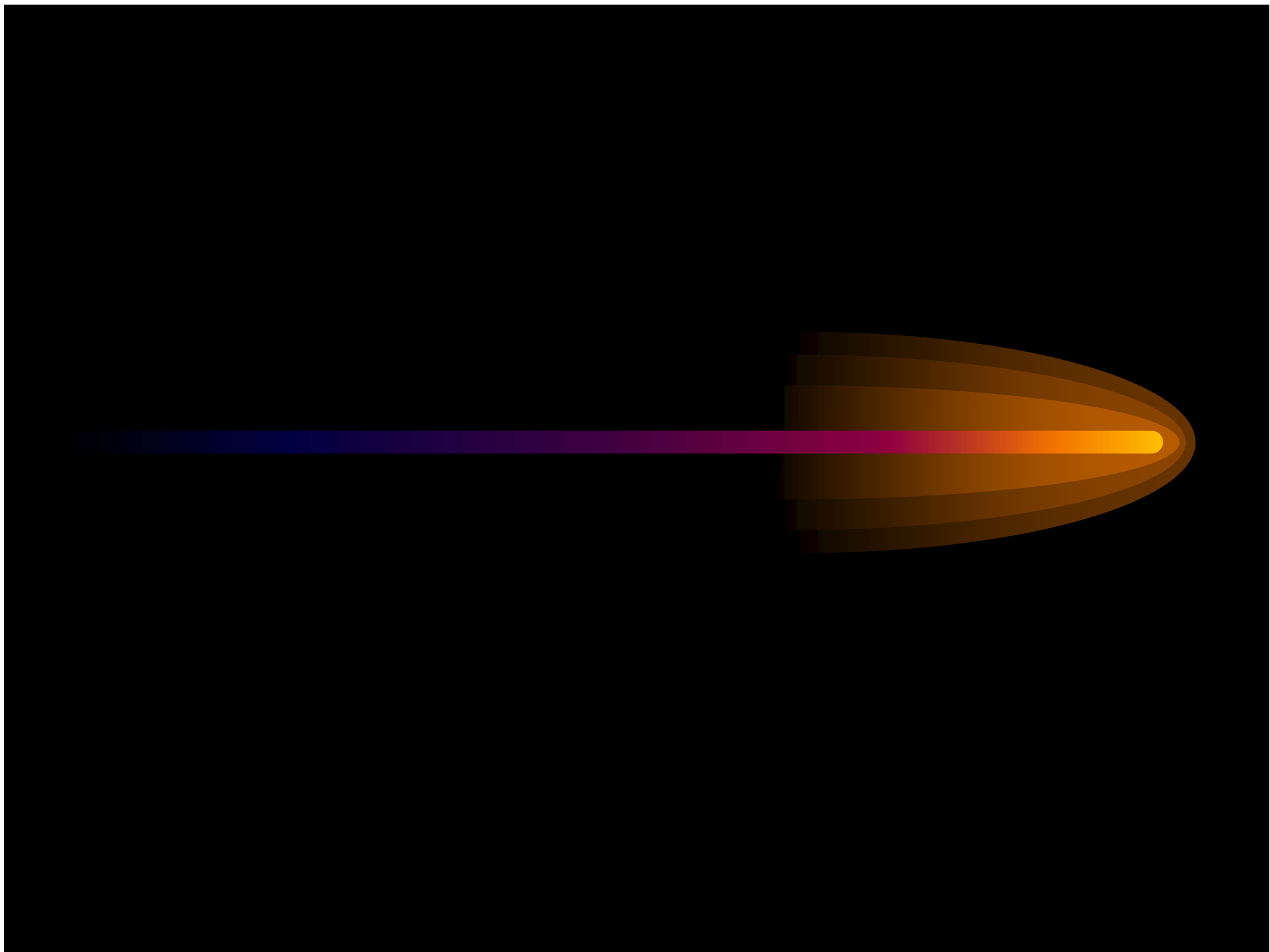
Stevens M, Tabrizi S, Garland SM. HPV genotyping using a modified Linear Array detection protocol. J Virol Methods 2006

Temporal trends of HPV types in cervical cancer (1920–2005)



Natural History



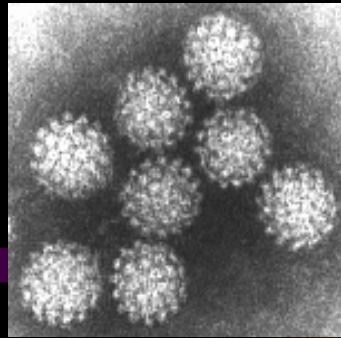


Cervical Cancer

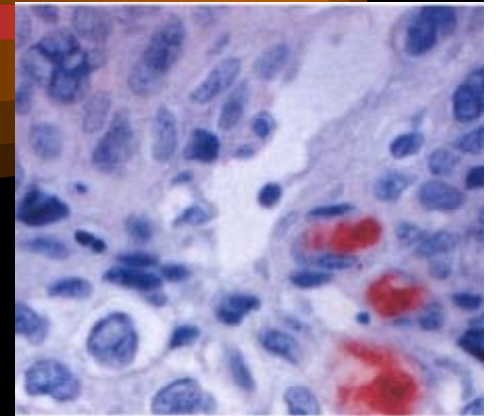
- Highly preventable disease!
 - **Screening & early detection**
 - Implemented early to detect (Pap smear) / treat cancer precursors.
 - Availability of molecular tests to increase sensitivity of detection.
 - **Vaccines**
 - Two vaccines available
 - ↓ incidence of HPV-16 infection and related dysplasia
 - Prevents HPV residing in genital tract (↓ transmission)

Detection by microscopy

- Electron Microscopy

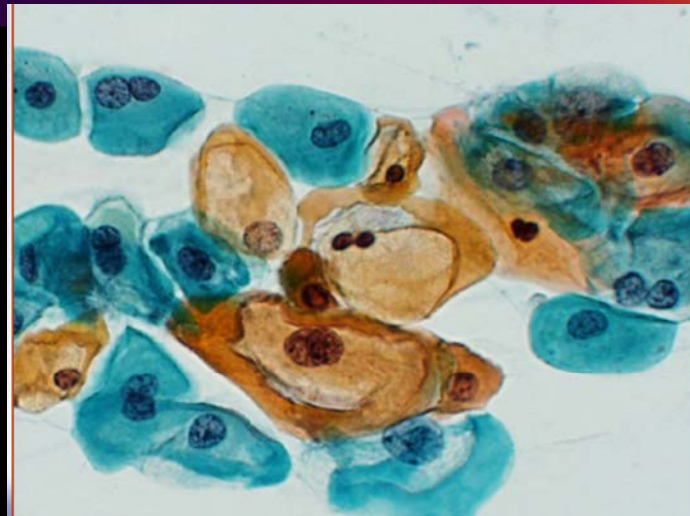


- Immunohistochemistry
(group specific antigen)

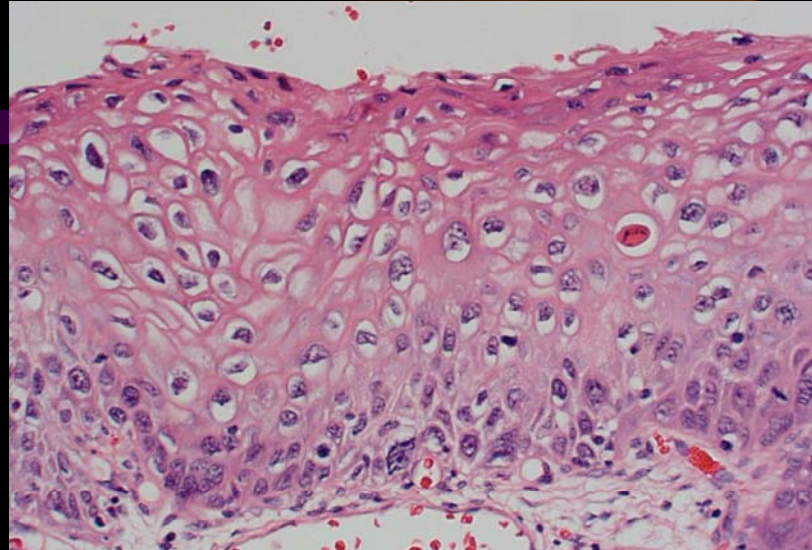


Detection by Microscopy

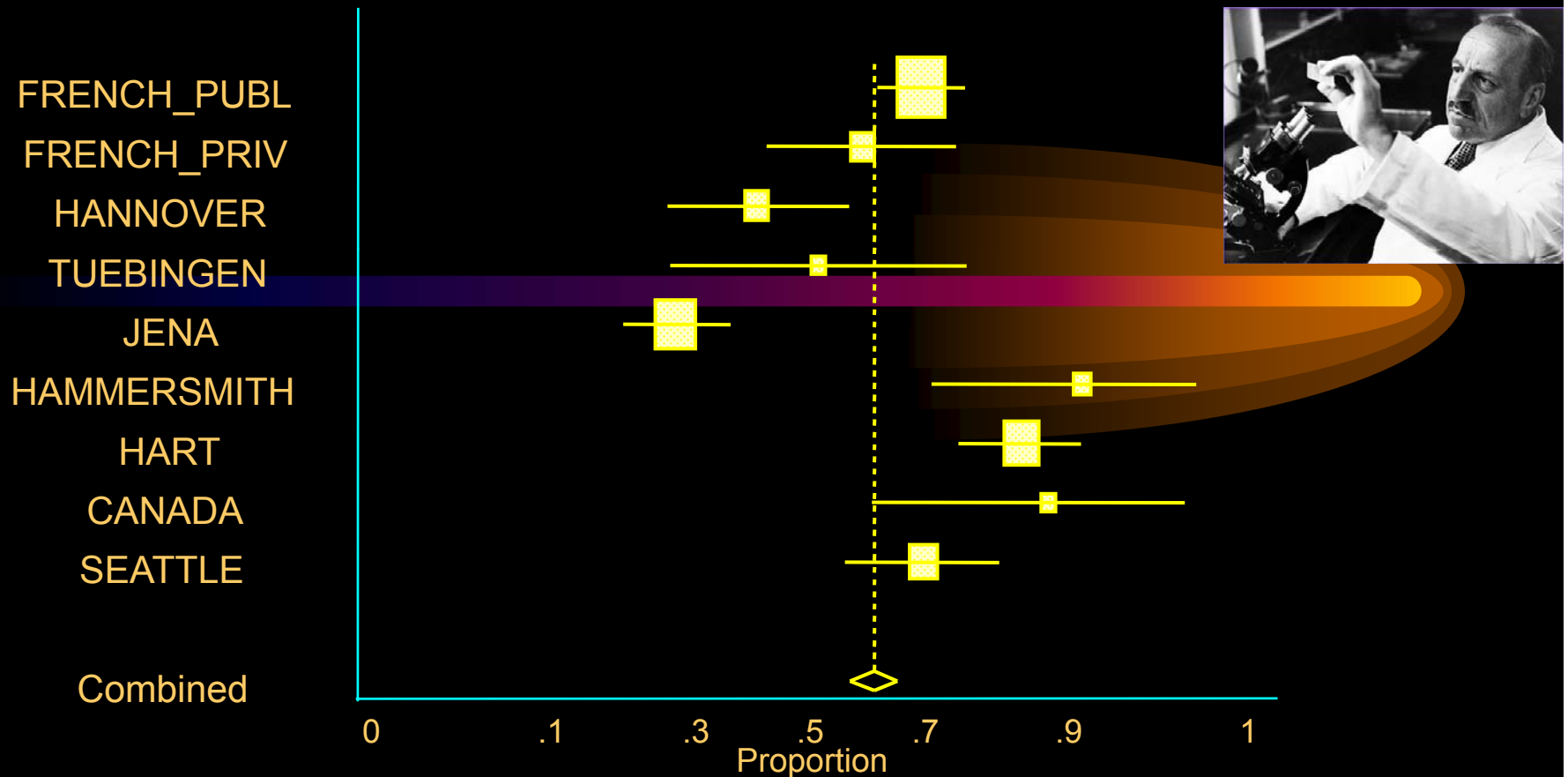
- Pap smear - treat cancer precursors.



- Histology



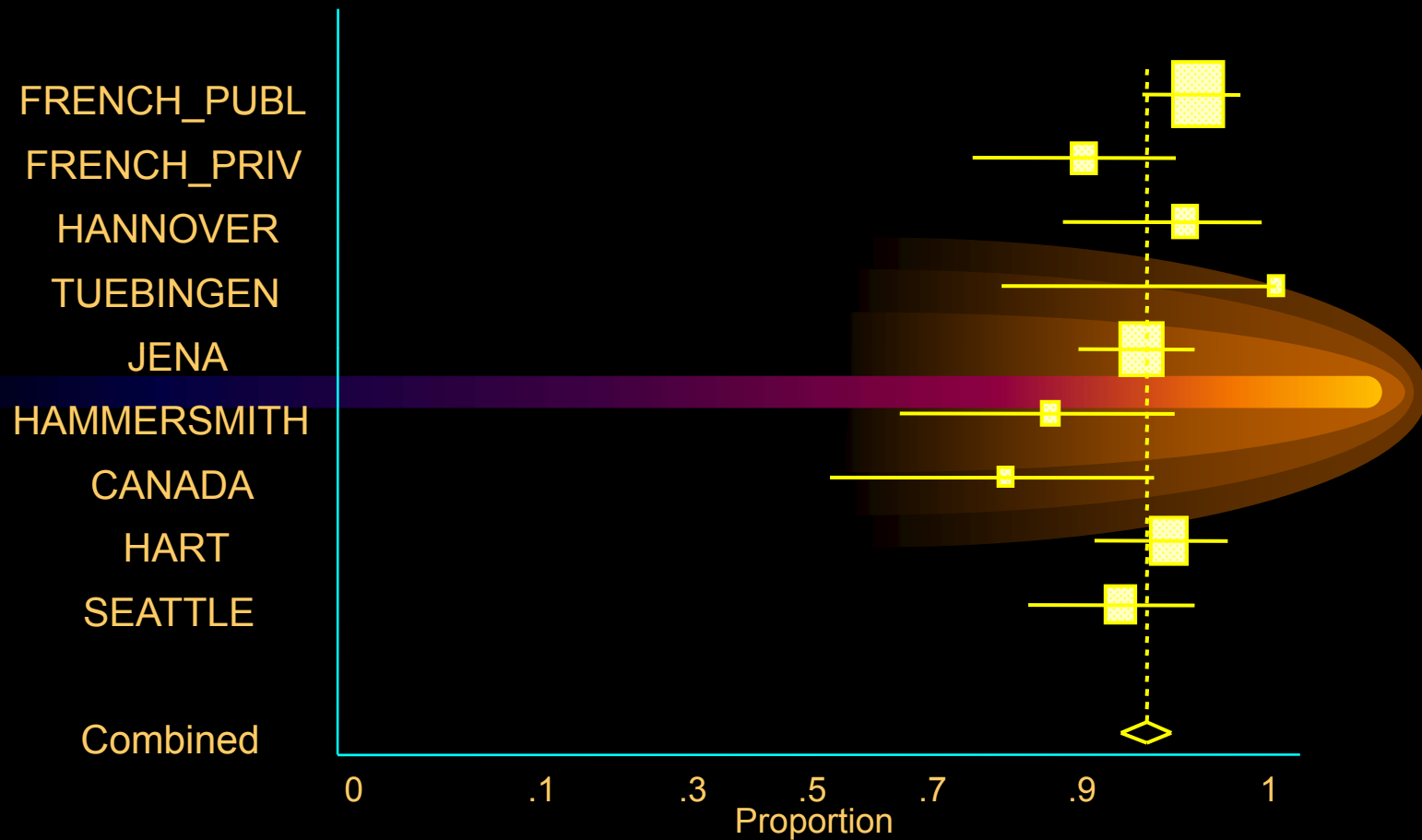
Pap Positivity for CIN2+ Histology



Sensitivity of Pap 53% [95% CI wide]

Cuzick J et al Int J Cancer 2006 119 European Nth American studies HPV primary screening

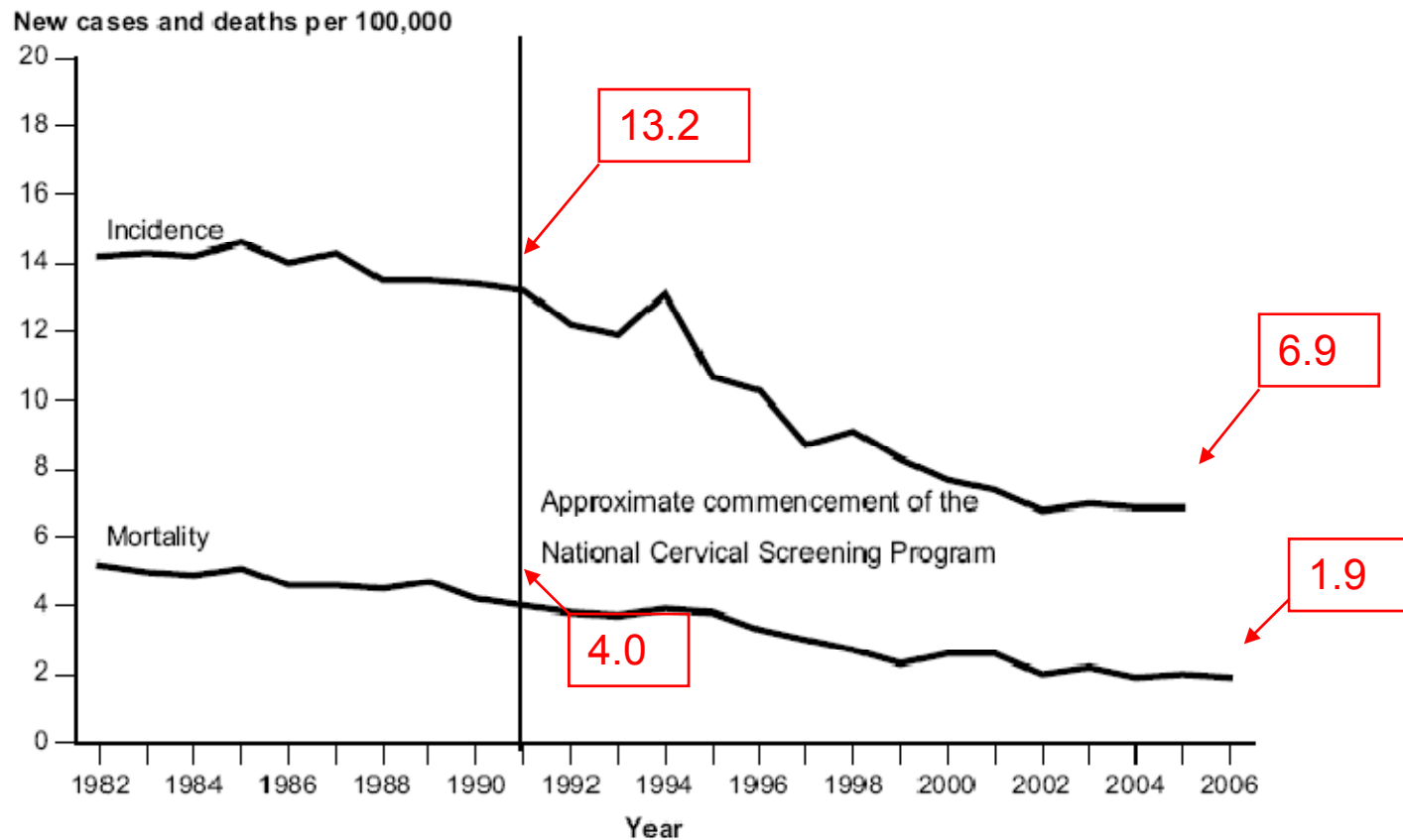
HPV Positivity for CIN 2+ Histology



Sensitivity of HPV DNA 96 % [95% CI tight]

Cuzick J et al Int J Cancer 2006 119 European Nth American studies HPV primary screening

Impact of Pap screening




Sources: National Cancer Statistics Clearing House and National Mortality Database, AIHW.

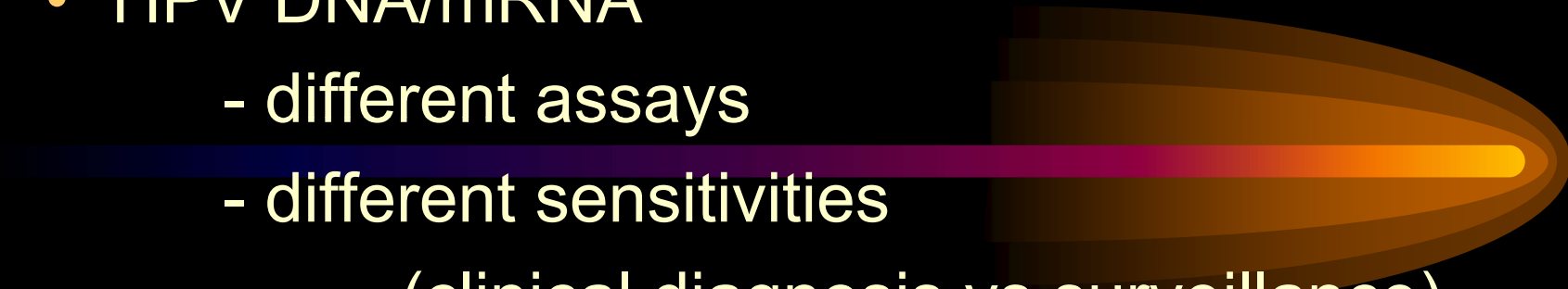
Figure 12.3: Incidence and mortality rates of cervical cancer in women of all ages, 1982–2006

Source: AIHW (Australian Institute of Health and Welfare) & AACR (Australasian Association of Cancer Registries) 2008. Cancer in Australia: an overview, 2008. Cancer series no. 46. Cat. no. CAN 42. Canberra: AIHW.

Detection by Serology

- Not yet diagnostic
 - Virus Like Particles
 - non-infectious analogs of a pathogenic virus
 - VLP- L1 assemble *in vitro*
 - VLP production not standardized
 - Different expression systems, preparative methods, QC approach
 - Formats vary (direct vs. indirect)
 - No gold-standard for setting threshold for positive result
 - Few inter-laboratory comparisons
- 

Detection by molecular biology

- HPV DNA/mRNA
 - different assays
 - different sensitivities
(clinical diagnosis vs surveillance)
- 

Commercial HPV tests (1)

- Hybrid Capture 2 Qiagen
- Care HPV Qiagen
- Luminex HPV assay Qiagen
- Consensus HPV typing kit Qiagen
- Amplicor HPV Test Roche
- COBAS 4800 HPV test Roche
- NucliSENS EasyQ HPV Biomerieux
- Aptima Gen-Probe
- Cervista Hologic (Third Wave Tech)
- BIOPAP QTS HPV Kit Loxo
- Abbott RealTime High Risk HPV , Abbott
- AID STD assay GenID
- AID HPV screening kit GenID
- AID HPV typing kit GenID

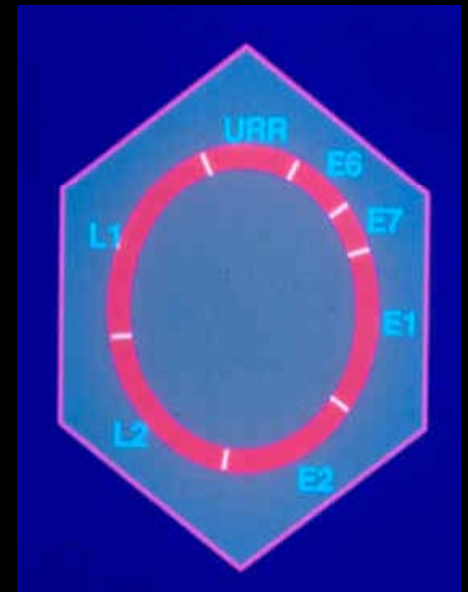
Commercial HPV tests (2)

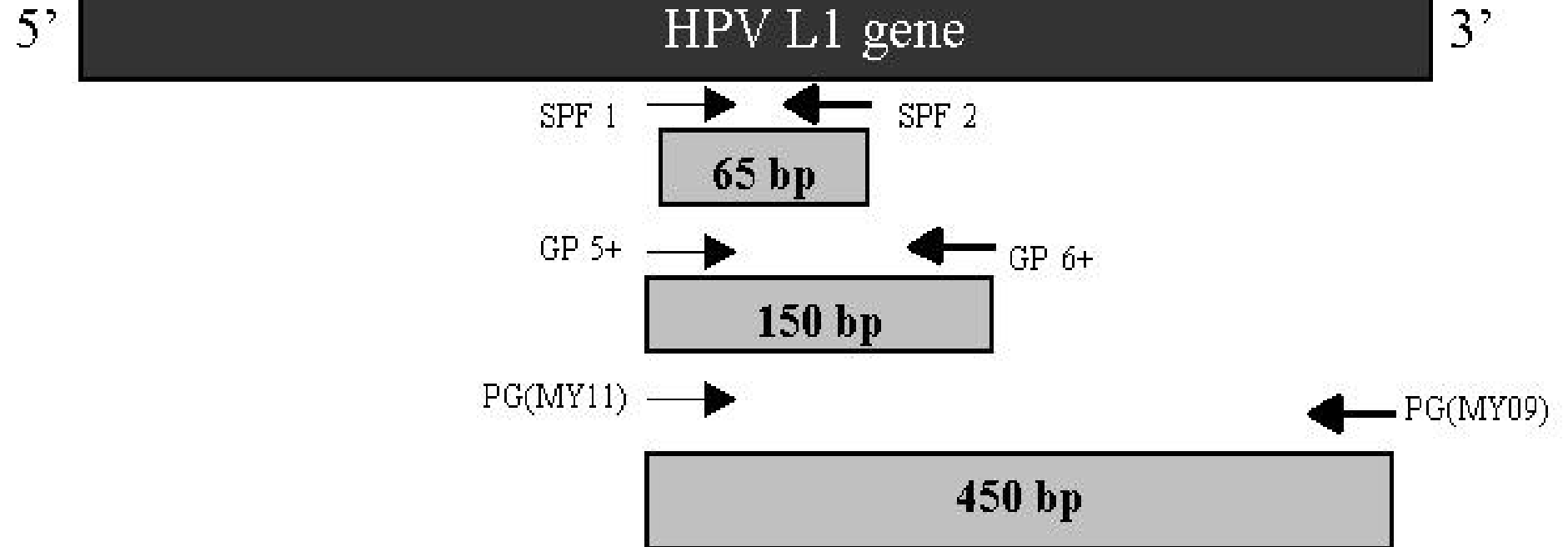
- Linear ArrayExtra HPV Genotyping Kit Innogenetics
- PCR Human Papillomavirus Detection Set Takara
Mirus Bio
- Array Papillomavirus Genomica
- ProDect Chip HPV typing Bcs Biotech S.P.A
- PapType Genera Biosystems
- LCD Array HPV 3.5 Chipron
- Seeplex HPV Genotyping Seegene
- Viroactiv Virofem
- HPV OncoTest Invirion Diagnostics
- Genpoint Tm HPV test Dako-Oxoid
- Reveal HPV Real-Time HPV Detection Kit GenoID
- Luminex HPV Genotyping, Multimetrix/Progen
- Papillocheck, Greiner BioOne

Etc

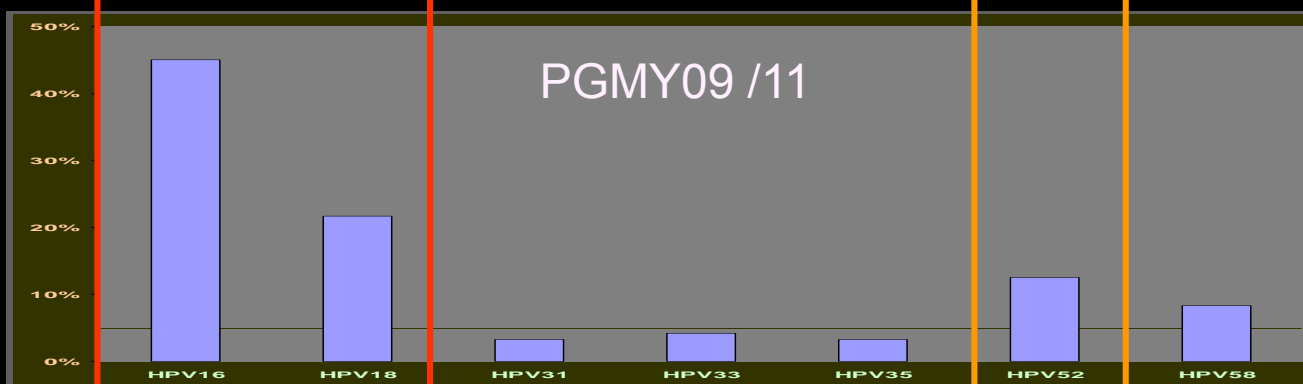
Polymerase Chain Reaction

- Very sensitive about 1-10 copies /100,000 cells
- Allows testing of various sample types
- Allows testing of archival samples with poorer quality DNA
- Consensus assays
 - generally target **L1 region**
 - conserved
 - primers vary:
MY09/MY11, SPF1/SPF2
PGMY09/PGMY11, GP5+/GP6+
 - detection systems vary:
ELISA, line blot





Ranking of HPV types in Hong Kong



HPV 16 HPV 18 HPV 31 HPV33 HPV 35 HPV 52 HPV 58

Why QA for HPV?

- Diverse range assays
- Different sensitivity and specificity
- Demand
 - ↑ requests for testing
 - ↑ labs performing testing
 - ↑ new tests for HPV testing
- QAP currently available in Australia through RCPA

WHO HPV Lab Network

Centres for surveillance/monitoring in areas matching ambitious vaccine introduction



● Selected 9 centres for strengthening lab facilities for HPV-DNA and antibody detection

What test to use?

CLINICAL:

- screening
- triage of equivocal/ borderline Paps
- monitor post dysplasia treatment

EPIDEMIOLOGY:

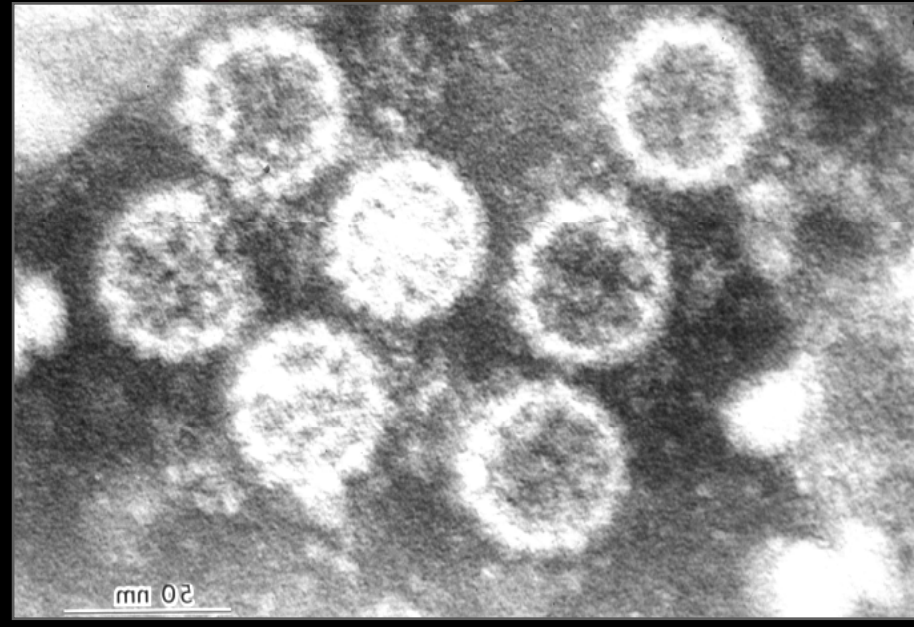
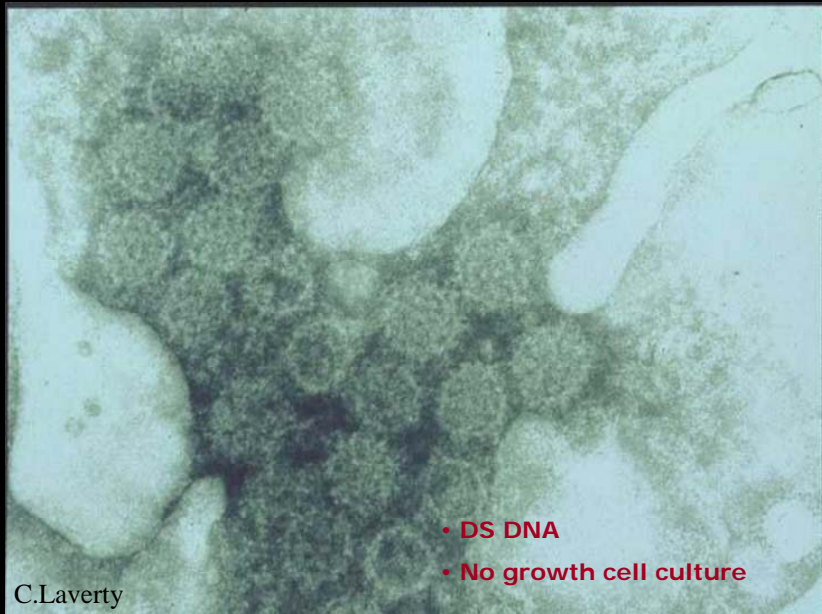
- surveillance geographical areas
 - HPV prevalence
 - HPV genotype prevalence

VACCINE:

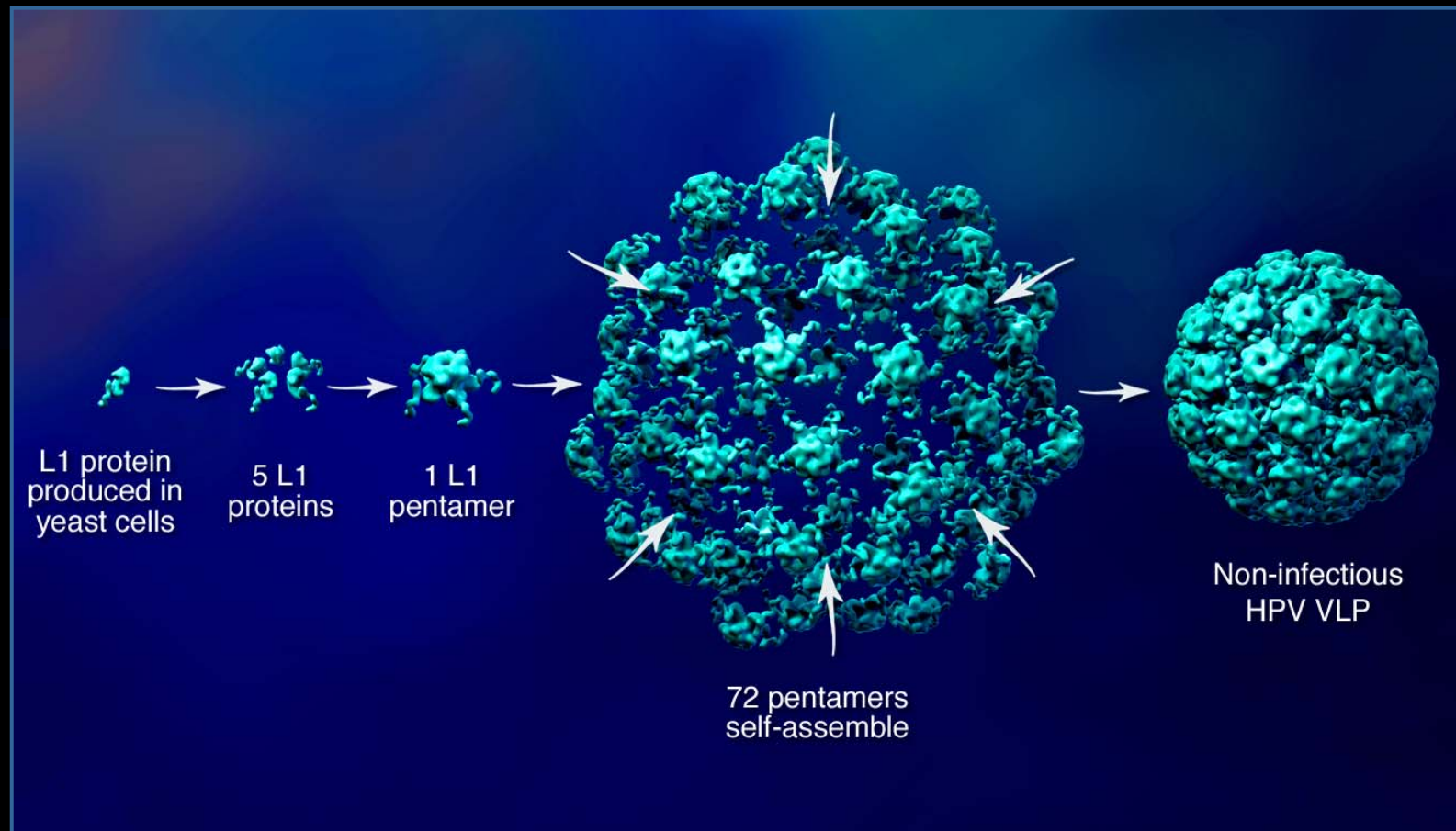
- pre and post vaccine implementation
 - vaccine efficacy trials
 - different regions worldwide

Virus Like Particles

- Described in 1990
- Resemble the virus physically and immunologically



HPV prophylactic vaccines: L1 protein self-assembles into VLPs¹⁻⁴



1. Berzofsky JA, et al. *J Clin Invest.* 2004;114:450–462.
2. Kirnbauer R, et al. *Proc Natl Acad Sci USA.* 1992;89:12180–12184.
3. Modis Y, et al. *EMBO J.* 2002;21:4754–4762.

- **highly immunogenic**
- **elicit type-specific antibodies that are neutralizing**

Currently licensed prophylactic HPV vaccines: product characteristics

	BIVALENT CervarixTM	QUADRIVALENT Gardasil[®]2
Antigen	VLPs of HPV 16 & 18	VLPs of HPV 16, 18, 6 & 11
Adjuvant	AS04 (Al(OH) ₃ + MPL)	AHHS
Expression system	Baculovirus expression vector	Yeast
Administration	0, 1 & 6 months by intramuscular injection	0, 2 & 6 months by intramuscular injection

1. CervarixTM. European Summary of Product Characteristics, 2007;

2. Gardasil[®]. European Summary of Product Characteristics, 2008.

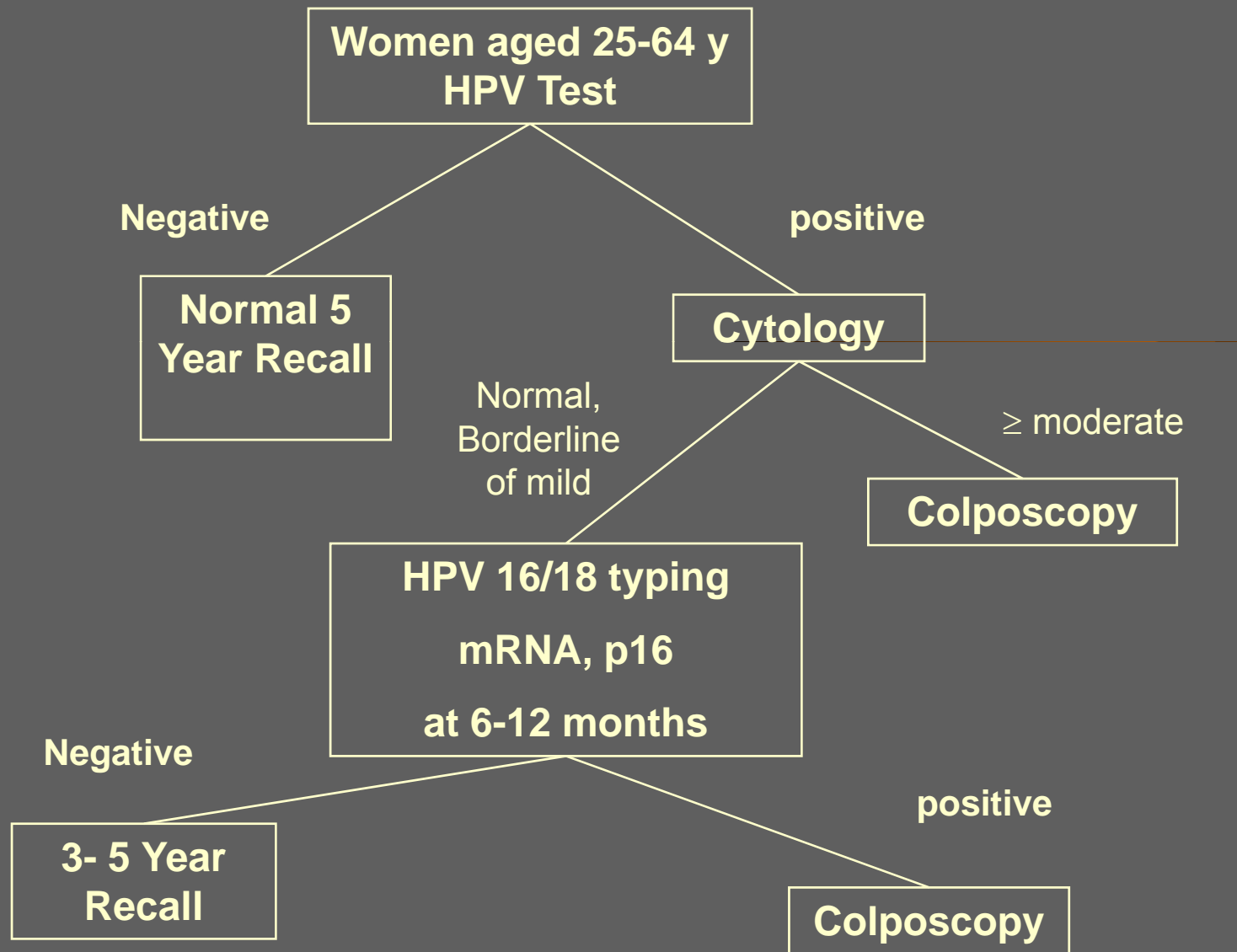
Potential changes to screening program

- Reduction of HPV 16/18 which will reduce HSIL
- Reduction of HSIL will lead to reduced exposure for reading Pap slides for training purposes
- Cost effectiveness and effectiveness of cervical screening is likely to change over time

Some potential possible changes to screening program

- Primary HPV DNA testing
 - Use of validated assays in screening population
 - Use of HPV typing (16/18 positive) or other progression markers
 - Allows further extension of pap interval
 - Cytology triage
- Use of new cytology technology
 - Automated image analysis with LBC

Potential Screening Algorithm

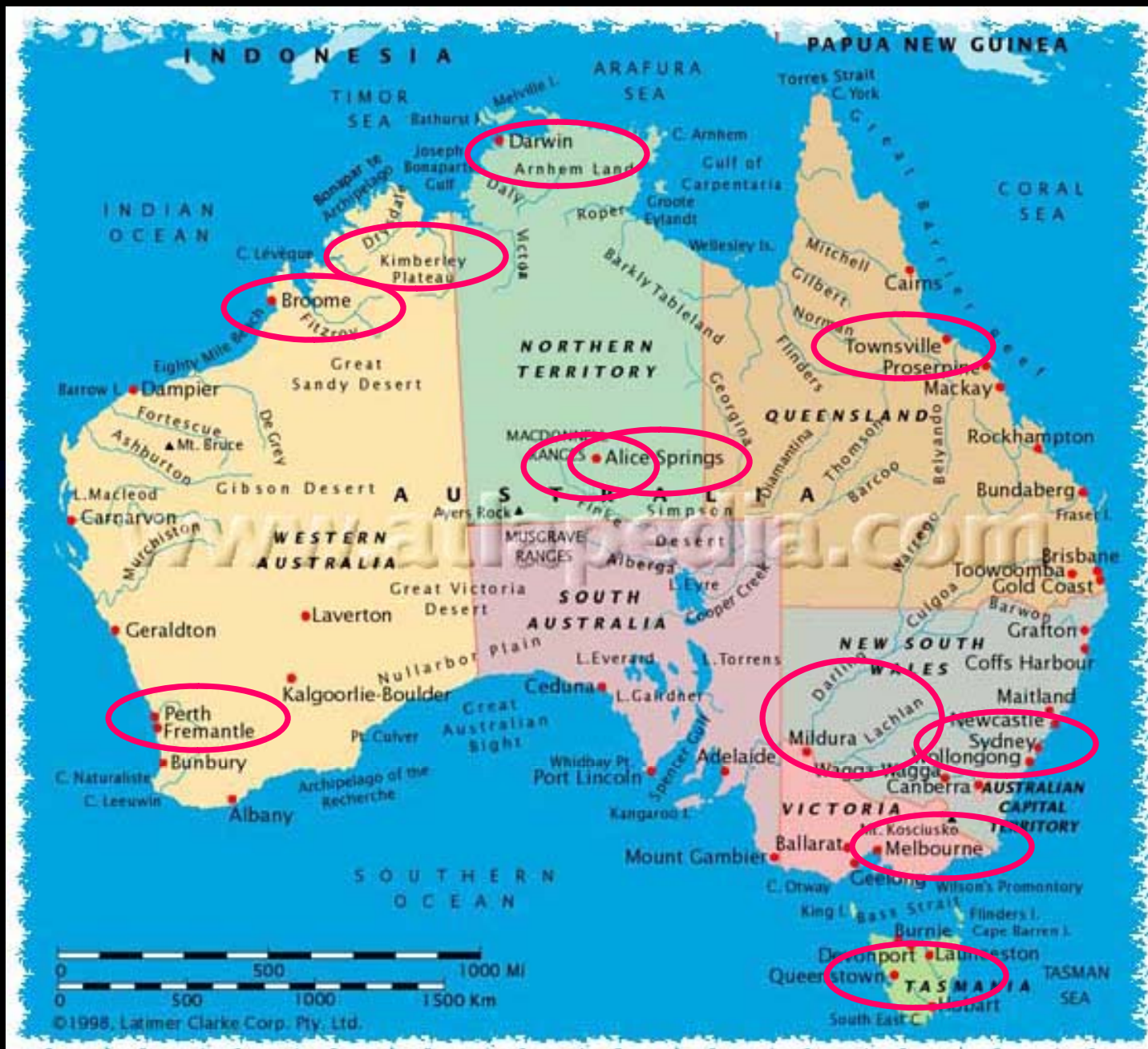


Implications of vaccines for HPV prevention in population

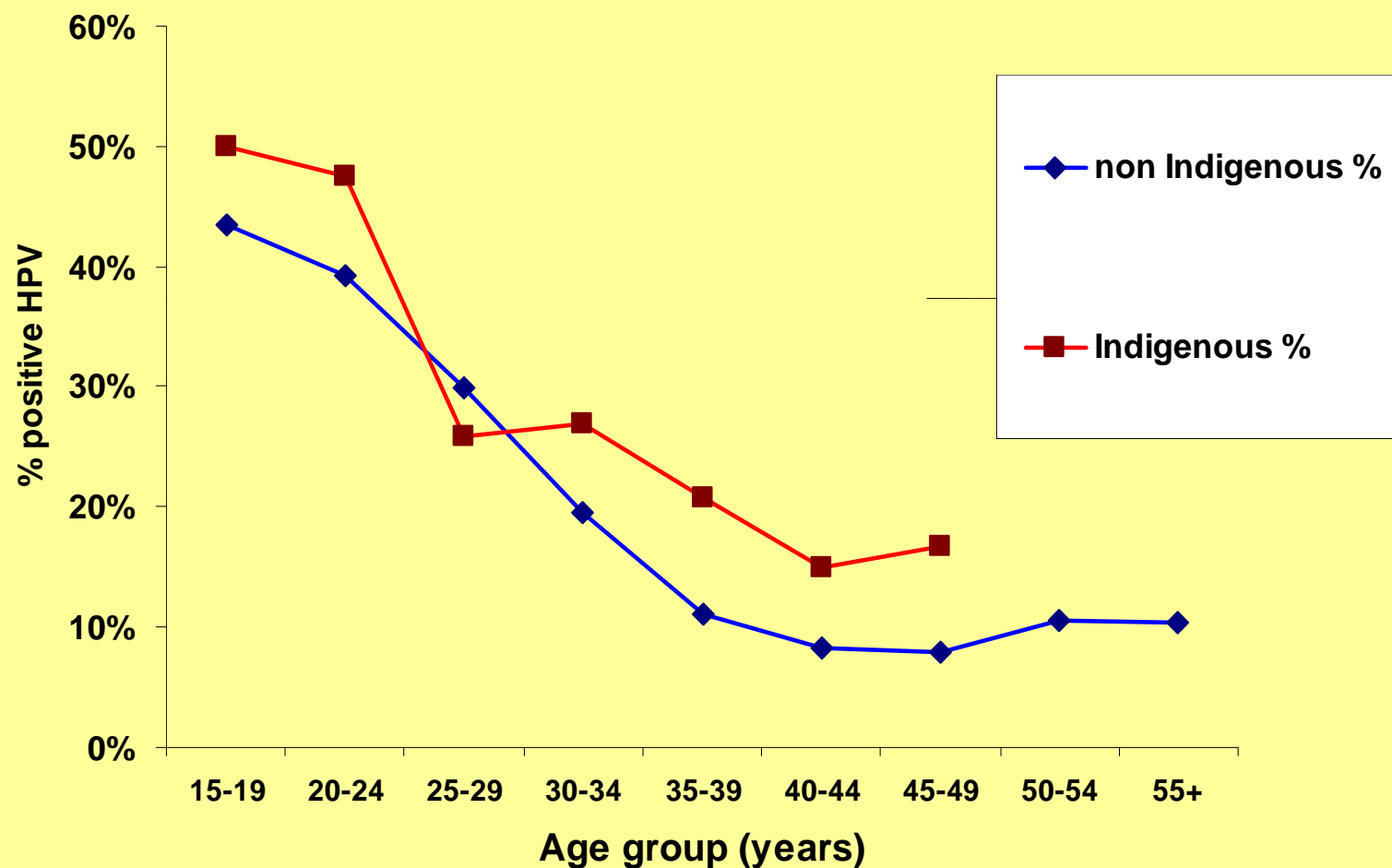
- vaccinating could prevent > 70% of invasive cervical cancers and HPV-related disease
- Issues: proportion of disease burden caused by various genotypes in different regions?
- Replacement as vaccine types eliminated?

Research Plan

1. To estimate the prevalence of type specific genital human papillomavirus (HPV) infection prior vaccine:
 - in the Australian female population
 - by age group
 - Indigenous status
 - cervical (Papanicolaou or "Pap") smear status
 - region of residence
2. To assess the potential impact of an HPV population vaccination strategy through the use of disease modelling



Percentage of women positive for HRHPV by Indigenous status and age group



None of the differences by age group are statistically significant

HPV 16 and 18 positivity by Indigenous status and age group

