



Assessment of Human Papilloma Virus (HPV) Vaccine Antibody Response by Pseudovirus-based Neutralization Assays

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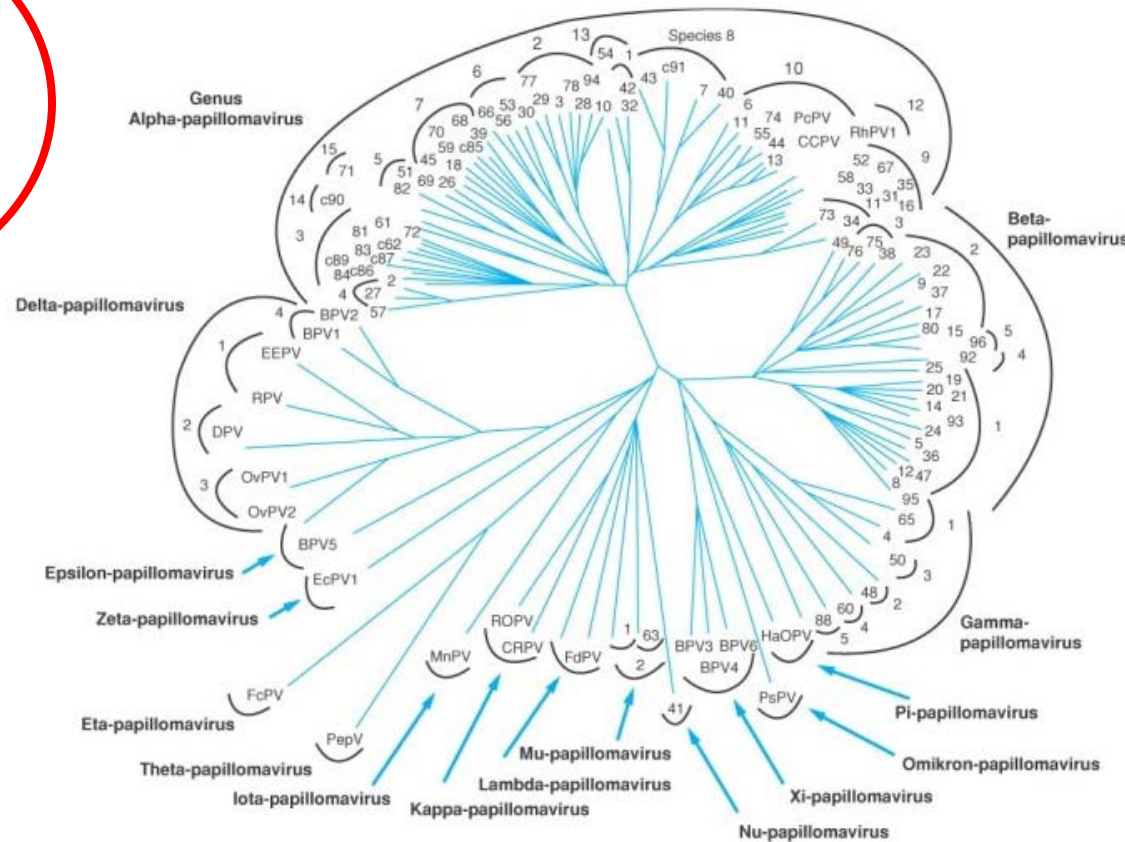
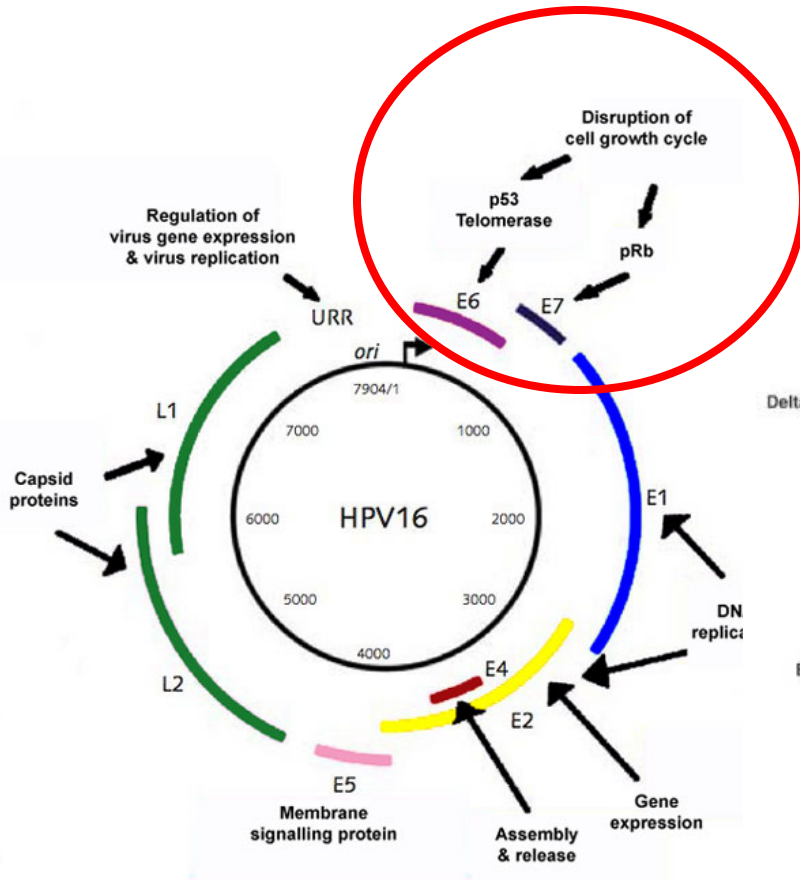
Objectives

1. Provide an overview of HPV pathogenesis and disease burden
2. Describe the principle behind HPV genotype-specific Viral Like Particle (VLP) based vaccines
3. Illustrate how VLP's and Pseudoviruses can be used to measure type specific antibody
4. Discuss challenges in measuring HPV vaccine induced immunity

Human Papillomavirus (HPV)

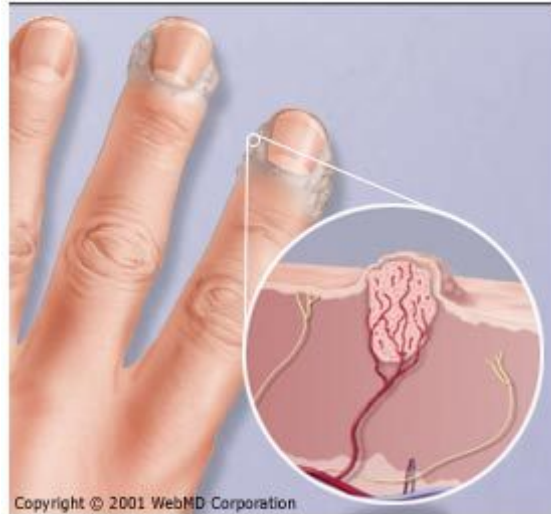
Double stranded closed circular DNA Virus

>130 genotypes



Pathogenesis/Presentation - Bad

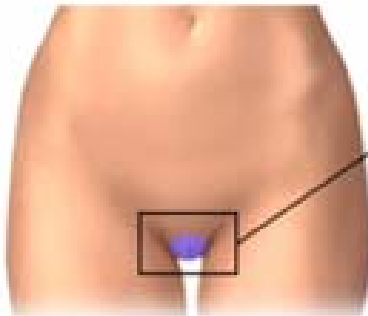
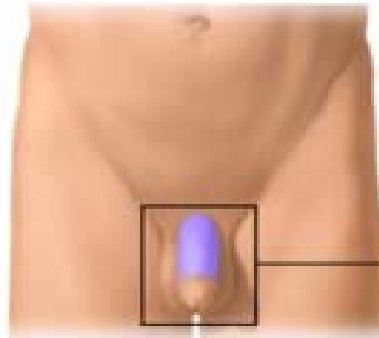
Warts



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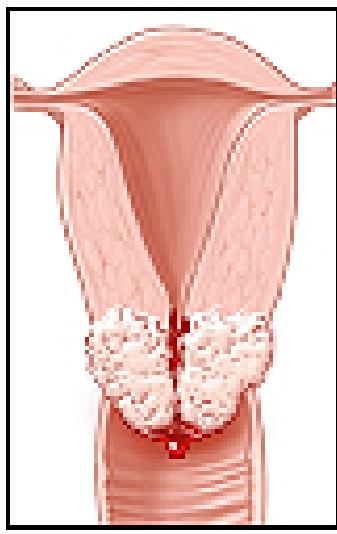
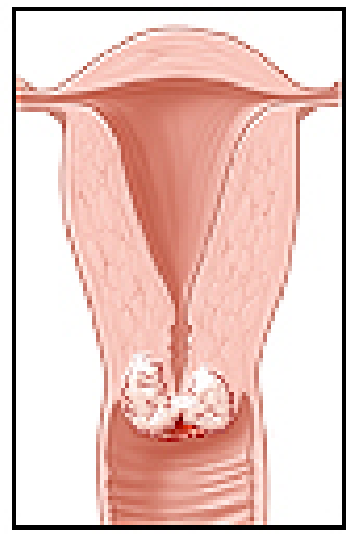
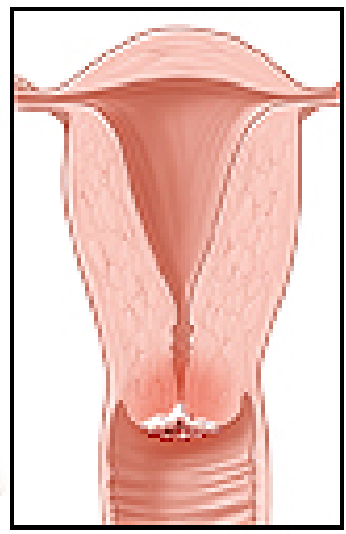
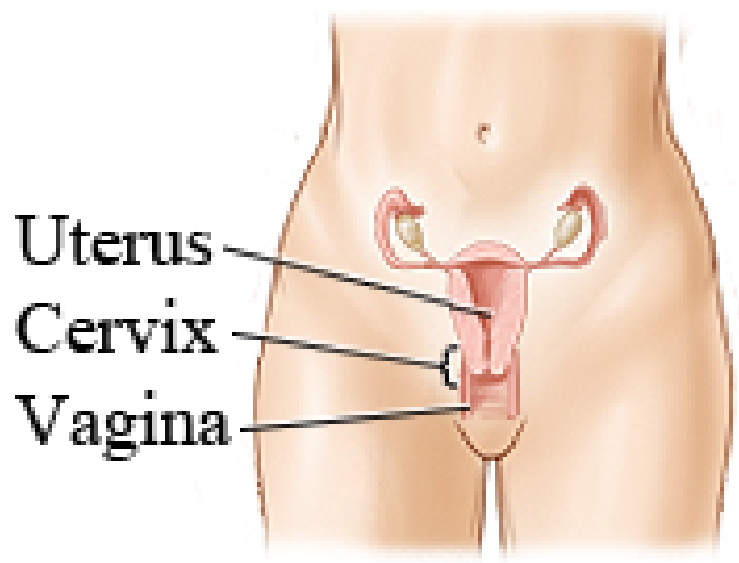
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Warts
condyloma

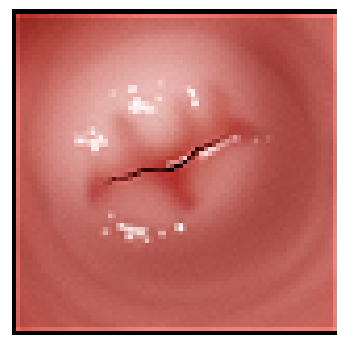


Genital warts:
Found on shaft of penis (male),
vagina, vulva, cervix (female)
and around anus

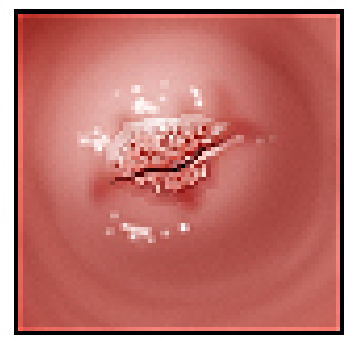
Pathogenesis/Presentation – Really Bad



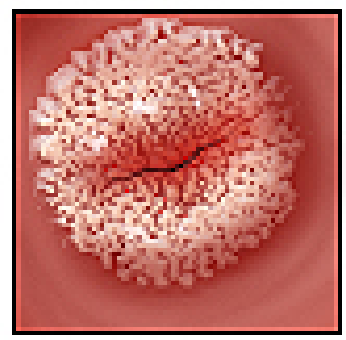
View of cervix as seen through the vagina



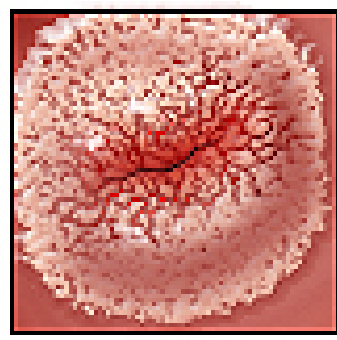
Normal



Early stage IB



Late stage IB

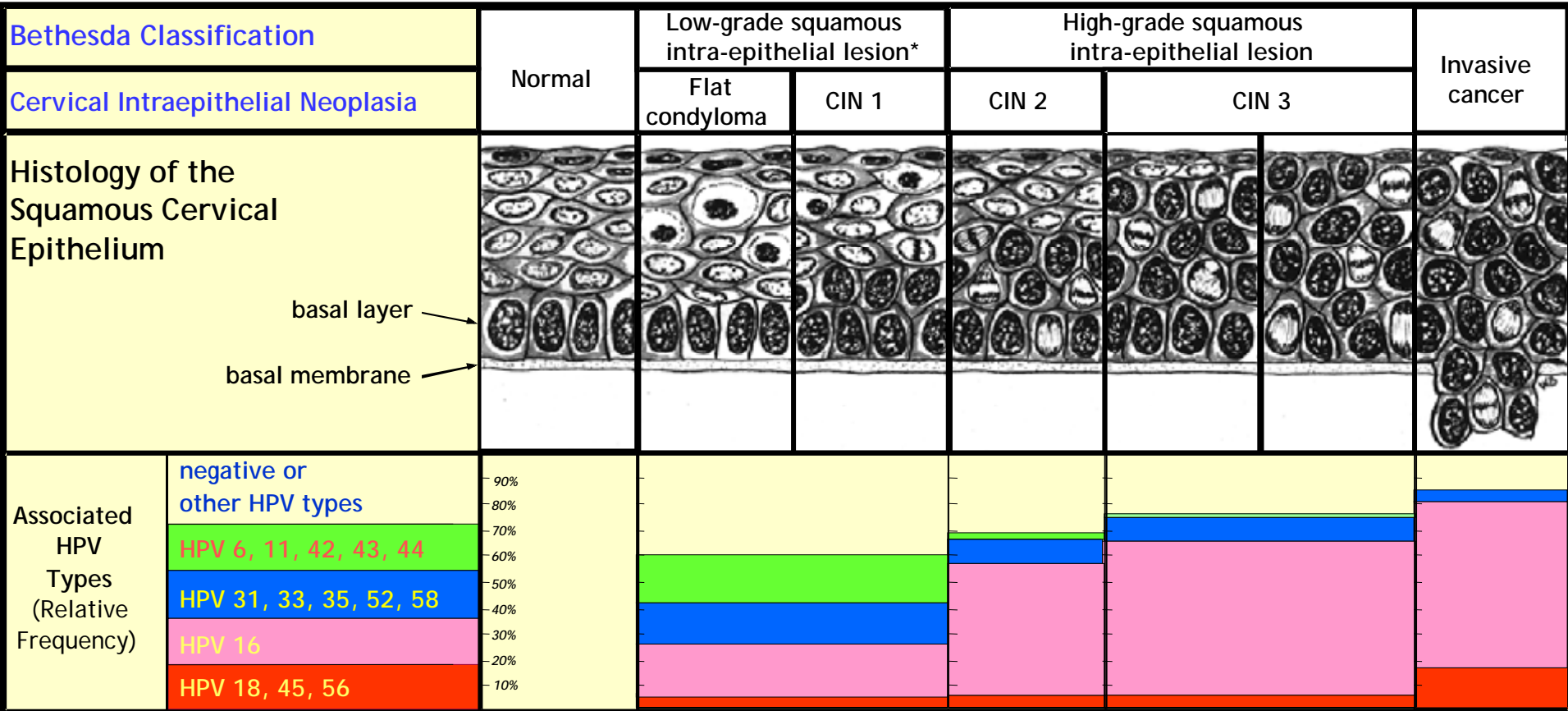


Stage IIB

HPV

- 13 to 16 HPV types are known to be oncogenic →
≈100% of cervical cancers, most rectal cancers and
≈66% of head and neck cancers (Frazer 2010;
Syrjänen 2007)
 - 70% of cervical cancers are due to HPV 16 & 18
 - 90% of genital warts are due to HPV 6 & 11 -
disfiguring, hard to treat
- Most individuals are infected with either a high-risk
or low-risk HPV during their lifetime and most
resolve their infection (Dunne et al . 2007)

Cervical Evolution from Condyloma to Cancer



The prevalence of high-risk oncogenic HPVs increases with the severity of the lesion

* LSIL also includes ASCUS (atypical squamous cells of unknown significance)

Modified from Bonnez W. Papillomavirus. In: Richman RD et al eds. *Clinical Virology*. 2nd ed. Washington, DC: American Society for Microbiology; 2002:557-596.

Good

- HPV L1 capsid proteins produced in cell lines → self assemble into Viral Like Particles (VLPs)
- Vaccination with VLPs induces strong type-specific neutralizing antibody
 - Lyengar et al, 1996
- VLP induced immunity protected against infection in a dog model
 - VLPs needed to be conformationally intact
 - Protection could be passively transferred
 - Suzich et al. 1995

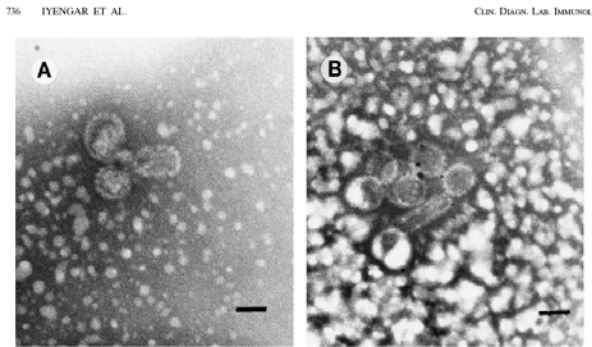
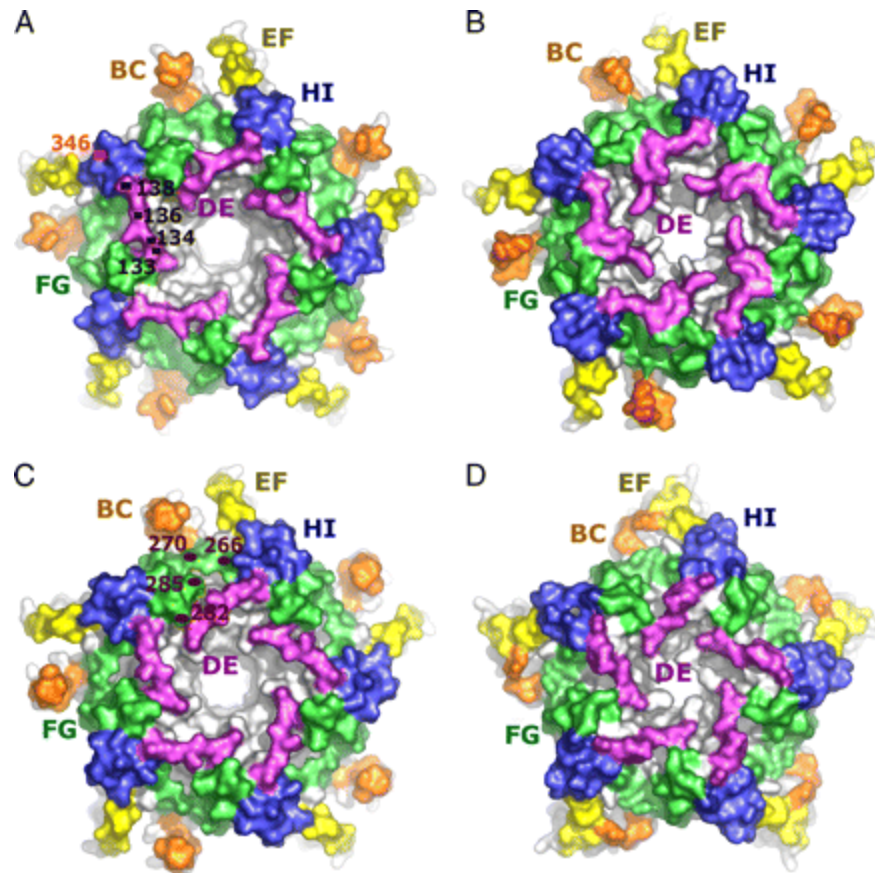


FIG. 3. Electron micrographs of in vitro translated HPV-16 L1 (A) and of baculovirus-expressed HPV-16 L1 (B). Bars, 50 nm.



Surface representation of L1 pentamers from four HPV types. The surface loops are colored differently on the surface: BC (*orange*), DE (*violet*), EF (*yellow*), FG (*green*), and HI (*slate*). A, HPV11; B, HPV35; C, HPV16; D, HPV18 L1. The distinct structural features can be observed for the surface loops among the four HPV types. Neutralizing epitopes are shown schematically on HPV11 and HPV16 pentamers. *Black square*, H11.F1 and H11.G5; *orange square*, H11.H3; *red ellipse*, H16.V5 and H16.E70 (Bishope et al. J Biol Chem 2007)

Preventative HPV Vaccines

- HPV infection is ubiquitous → only 50% to 60% of natural infections induce detectable Ab
 - Carter et al. 2000
- Natural infection induces a weak Ab response – Ab ≠ protection
- Notion of using VLP-based vaccines to induce a protective type specific immunity prior to exposure
- Two VLP-based vaccines (Gardasil® quadrivalent HPV 16, 18, 6 & 11; Cervarix® bivalent HPV 16 & 18) are licensed and a nine valent vaccine is being evaluated (Merck)
- >98% effective at reducing type specific associated cytologic abnormalities; preventing type specific associated CIN 1, 2, 3 as well as preventing vaccine type specific associated condyloma

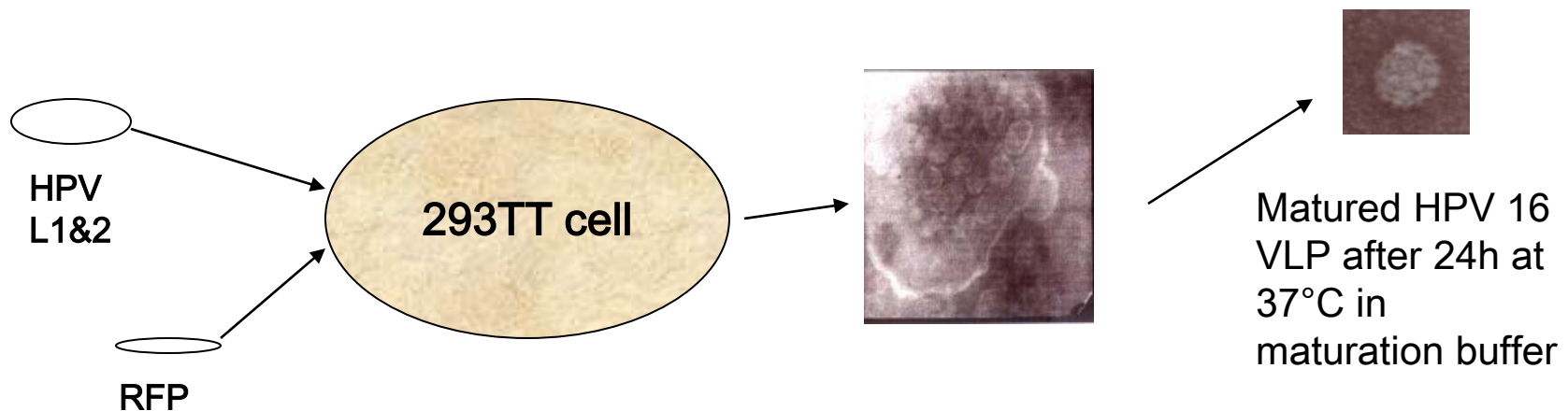


Ab as an Immune Correlate of Vaccine Protection

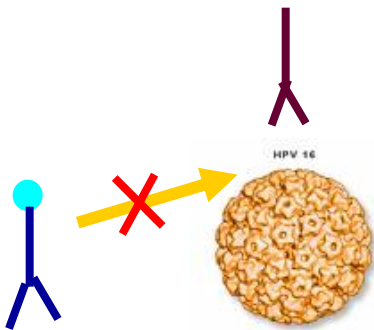
- Vaccine protection lasts at for at least 10 years but the durability is unknown!
- Protective immunity → likely relates to both CMI and Ab
- It is unknown whether the total amount, a particular type or specificity of Ab provides the best surrogate for protection
- Current assays are not standardized → not possible to use Ab induced/measured by different vaccine products to compare efficacy
- Goal is to develop and validate the best surrogate markers for protection → Ab is often selected because it is “easy” to measure

What is a Pseudovirus (PsV)?

- HPV does not easily replicate in cell lines → traditional neutralization assays are not feasible → solution was developed by Buck et al. (2005)
- The HPV genome encodes 2 structural proteins, L1 and L2
- These genes are incorporated into a plasmid with the appropriate promoters
- Transfection of 293TT cell lines results protein expression and self-assembly of the L1 & L2 proteins into empty HPV capsids
- Capsids can incorporate plasmids of ~ 8 kB and therefore can carry a reporter gene such as one encoding Secreted Embryonic Alkaline Phosphatase (SEAP) or Red Fluorescent Protein = PsV

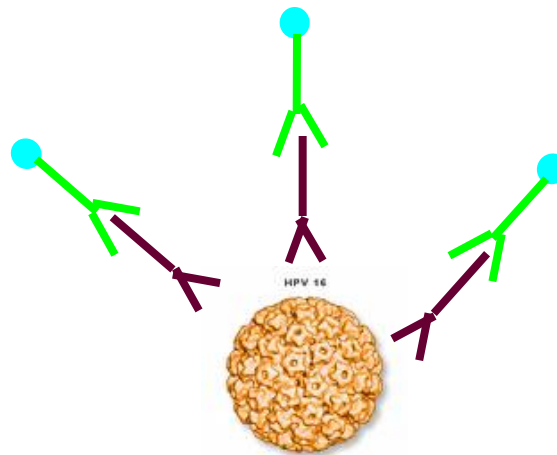


Merck Luminex-based competitive immunoassay (cLIA)



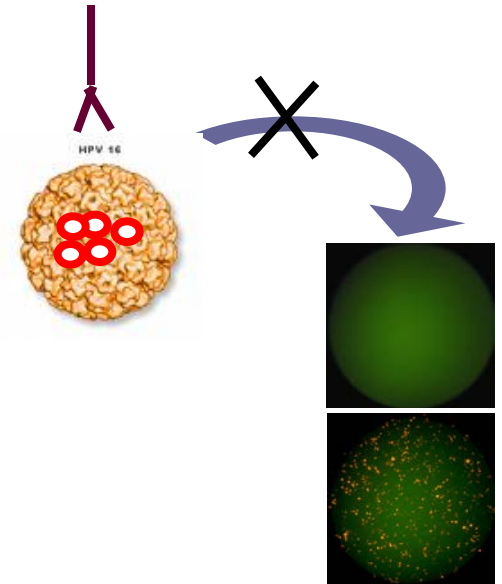
- Labelled Ab binds known neutralizing HPV VLP (L1) epitopes – surrogate for neutralization
- Decreased signal in presence of host Ab
- Multiplex

Total IgG e.g. Luminex-based LIA or ELISA



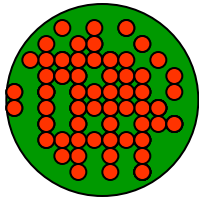
- Does not differentiate neutralizing from non-neutralizing Ab
- Plate-based ELISA
- Luminex bead-based multiplex assay

PsV Neutralizing Ab Assay



- Pseudovirus based neutralization assay
- Developed by NIH – type specific VLP's L1 & L2 produced in 293TT
- VLP is packaged with a reporter gene – SEAP/GFP/RFP
- Need a well/HPV type

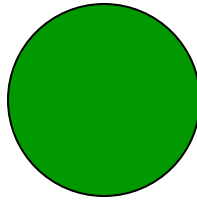
Control



100 RF Cells

**No Ab
or Ab
diluted
to
extinction**

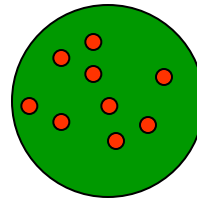
100% NT



No RF Cells

**Complete
neutralization
least amount
of Ab to
completely
neutralize**

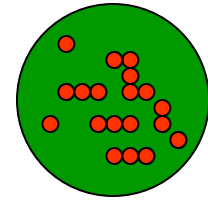
90% NT



1-10 RF Cells

**90%
neutralization
but not
enough to
neutralize all
the PsV**

Partial



> 10 < 90 RF Cells

**Detectable
neutralizing
Ab**

Challenges in Measuring HPV Post Vaccination Ab

- Among individuals who initially sero-convert after Gardasil® vaccination, HPV antibody levels measured by the Merck Competitive Luminex Immunoassay (cLIA) may decline to undetectable within as little as 2 years
- In particular for HPV 18, by 42 months, 20% to 40% can be cLIA negative, despite continued vaccine efficacy

Example

- At month 24, 620/620 (100%) were still sero-positive for HPV 16 and 589/620 (95%) for HPV 18 by cLIA
- Of the 31 HPV 18 cLIA negative subjects, PsV NAb was detected at the following endpoints
 - 31/31 (100%) NTpartial
 - 13/31 (42%) NT90
 - 6/31 (19%) NT100
- All 31 HPV 18 cLIA negative subjects displayed some level of HPV 18 neutralizing Ab at month 24 and most also had Ab as measured by the Merck LIA T-IgG

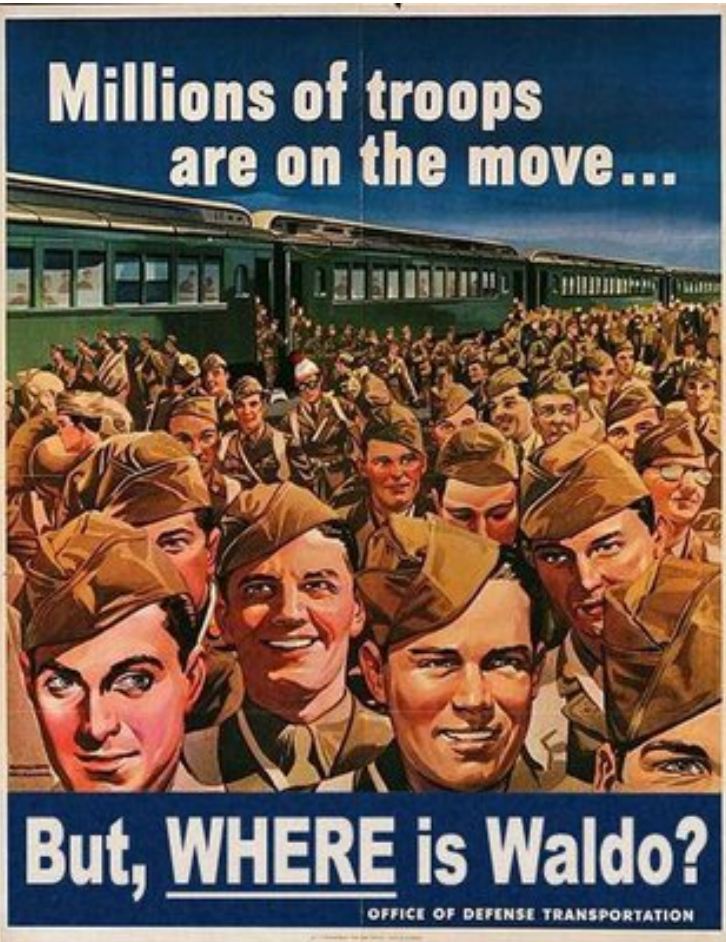
Conclusions

- Merck cLIA may not optimally detect Ab likely because of its monoclonal design
- Neutralization can occur from specific NAb or by steric interference with binding
 - PsV NAb Ab titre depends on the selected endpoint
- Merck LIA T-IgG assay detects a mixture of neutralizing and non-neutralizing epitopes → are these Ab appropriately specific?

Multiplex EIA Challenges

- Given the array of different HPV genotypes – what is the degree of cross reactivity & what are relevant interactions within a multiplex assay?
- Neutralizing Ab has been shown to be important for protection – how important are Ab's that might cross react between HPV types?
- How does one standardize across an array of HPV types given that individuals may be exposed to multiple types during their lifetime and do these exposures affect vaccine induced Ab titres?

Questions



- Standardization remains an issue - which assay measures the right Ab(s) and what are protective levels?
- How does Ab durability correlate with vaccine protective efficacy?
- What is the relationship between Ab and other immunological control mechanisms?