Human Papillomavirus (HPV) Diagnosis

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Cervical Cancer

• 2nd most common cancer of women worldwide
  – ~ ½ 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

- 227 deaths
- ~ 700 cervical cancer cases - 13th
- 14,500 high grade abnormalities
- 16,500 low grade abnormalities
- 100,000 abnormal Pap smears
- ~ 2 million women screened

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
<table>
<thead>
<tr>
<th>Cancer</th>
<th>Relative Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung Cancer- smoking</td>
<td>10</td>
</tr>
<tr>
<td>Liver Cancer- HCV</td>
<td>20</td>
</tr>
<tr>
<td>Liver Cancer- HBV</td>
<td>50-100</td>
</tr>
<tr>
<td>Cervical Cancer- HPV</td>
<td>300-500</td>
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</tbody>
</table>
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 oncprotein
    - 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.


Beijing IPV 2007, abstract 4B-05
**Natural History**

- **Transient Infection**
  - HPV infection
  - Initial infection
  - Normal cervix
  - HPV-infected cervix
  - Clearance
  - Mild cytologic abnormalities

- **Persistent HPV Infection**
  - Progression
  - Regression
  - Precancerous lesion
  - Invasion
  - Cancer

**Spontaneous Regression Rate**
- 40%
- 20%
- 1%
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

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

P. Chan et al, Int J Cancer. 2005 Jul 19
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/ border-line 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

- DS DNA
- No growth cell culture
HPV prophylactic vaccines: L1 protein self-assembles into VLPs\textsuperscript{1–4}

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

Currently licensed prophylactic HPV vaccines: product characteristics

<table>
<thead>
<tr>
<th></th>
<th>BIVALENT Cervarix™¹</th>
<th>QUADRIVALENT Gardasil®²</th>
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<tbody>
<tr>
<td><strong>Antigen</strong></td>
<td>VLPs of HPV 16 &amp; 18</td>
<td>VLPs of HPV 16, 18, 6 &amp; 11</td>
</tr>
<tr>
<td><strong>Adjuvant</strong></td>
<td>AS04 (Al(OH)₃ + MPL)</td>
<td>AHHS</td>
</tr>
<tr>
<td><strong>Expression system</strong></td>
<td>Baculovirus expression vector</td>
<td>Yeast</td>
</tr>
<tr>
<td><strong>Administration</strong></td>
<td>0, 1 &amp; 6 months by intramuscular injection</td>
<td>0, 2 &amp; 6 months by intramuscular injection</td>
</tr>
</tbody>
</table>

1. Cervarix™, European Summary of Product Characteristics, 2007;
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

Women aged 25-64 y
HPV Test

Negative

Normal 5 Year Recall

Cytology

Normal, Borderline of mild

≥ moderate

HPV 16/18 typing
mRNA, p16
at 6-12 months

Negative

3-5 Year Recall

Positive

Colposcopy

Colposcopy

Cuzick
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

- Non Indigenous HPV 16
- Non Indigenous HPV 18
- Indigenous HPV 16
- Indigenous HPV 18

Percentage positive

Age group (years):
- 15-19
- 20-24
- 25-29
- 30-34
- 35-39
- 40+