Molecular detection of HPV

Aalst 2/12/2016

Prof. Dr. JP Bogers
Dr. I. Benoy
Conflict of interest

JPB is a pathologist...

JPB received travelgrants and researchgrants from Dako, Becton-Dickinson, Roche, Hologic, Innogenetics and MTM

JPB was PI for various company sponsored studies by Becton-Dickinson, Hologic, Innogenetics and MTM

JPB serves/served on the scientific advisory board of Roche, Hologic and GenProbe

JPB is medical director of AML, a private laboratory performing cytology and HPV testing

JPB is co-responsible of the National Reference center for HPV of Belgium
Primary cause of CxCa → persistent infection with hrHPV types
HPV: very common sexually transmitted virus
Most cervical HPV infections resolve spontaneously within 2 years.
Those that persist for 2 years or longer may lead to the development of precancerous lesions and CxCa.
It usually takes more than 10 years for precancerous lesions caused by HPV to develop into invasive CxCa.
CxCa is one of the leading causes of cancer incidence and death in women, most notably in low- and middle-income countries.
The Natural History of HPV Infection and Cervical Cancer

Human Papillomavirus

- Circular DNA virus
  → 7000 - 8000 base pairs
  → consisting of 72 pentavalent capsomeres
- Up to 200 different genotypes

- Divided into 3 regions
  Long Control region (LCR)
  Region of Early proteins (E1 to E5)
  Region of Late proteins (L1 and L2)

- Classified according to:
  Tropism: Cutaneous or Mucosal
  Risk of neoplasia: Low risk or High risk
HPV testing

Complicated by very low cancer/infection ratio

- Most (young) women can/will be positive at a specific time without relevant disease/increased risk
- Most infections will resolve spontaneously
- Counseling implications/ can cause stress
  - Paramount importance of clinician engaging in individual counseling, explaining the test and the relevance to each woman
  - HPV testing is perceived as a test for a sexually transmitted disease and not as risk stratification for cancer

Prevalence of hr-HPV-infection: age groups

<table>
<thead>
<tr>
<th>Age group</th>
<th>Prevalence</th>
<th>[95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 - 19</td>
<td>19.2</td>
<td>[12.8, 27.9]</td>
</tr>
<tr>
<td>20 - 24</td>
<td>19.2</td>
<td>[16.4, 22.3]</td>
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<tr>
<td>25 - 29</td>
<td>18.8</td>
<td>[16.5, 21.3]</td>
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<tr>
<td>30 - 34</td>
<td>14.5</td>
<td>[11.6, 18.0]</td>
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<tr>
<td>35 - 39</td>
<td>12.4</td>
<td>[9.4, 16.1]</td>
</tr>
<tr>
<td>40 - 44</td>
<td>10.7</td>
<td>[8.0, 14.2]</td>
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<tr>
<td>45 - 49</td>
<td>6.0</td>
<td>[3.9, 9.0]</td>
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<tr>
<td>50 - 54</td>
<td>10.5</td>
<td>[7.7, 14.1]</td>
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<tr>
<td>55 - 59</td>
<td>3.8</td>
<td>[3.6, 9.2]</td>
</tr>
<tr>
<td>60 - 65</td>
<td>4.5</td>
<td>[2.4, 8.1]</td>
</tr>
</tbody>
</table>

Pearson chi2(9) = 96.7931  Pr = 0.000
HPV detection

HPV’s can not be cultured *in vitro*

No serological tests available that are sufficiently sensitive for reliable detection of HPV presence

Currently used HPV detection assays rely on detection of viral nucleic acids (DNA, RNA) or viral proteins
HPV tests can be used for several distinct purposes

Identifying (wo)men at (cervical) cancer risk
- Detecting/predicting disease
- Follow-up

Epidemiological
- Assessing prevalence of type-specific HPV infections

Vaccine monitoring
- Determining protection against incident HPV infections
HPV detection

**HPV DNA tests**

Detect the viral DNA
Target = highly conserved region = consensus primers
Multiplex format

**HPV RNA tests**

Detect the viral mRNA
Target = mRNA coding for oncoproteins E6/E7
- Over-expressed in severe lesions and cancer
Active infection / cell transforming potential
CHOOSING THE RIGHT HPV TEST...

HPV TYPING

- No typing
  hr/lr
- Limited typing
  16/18/other
- Extended typing
  13 hr types/14 hr types
- Full typing
  > 14 HPV genotypes detected separately

HPV typing can be important for:
- risk assessment
- vaccination
- follow-up
L1 testing

+ Conserved region allows amplification of large number HPV genotypes in a single reaction
- Possibility to miss rare invasive CxCa where L1 is totally integrated (?)

Type specific E6/E7

+ integrated HPV types will not be missed
- competition for reagents, can lead to false negative results for multiple type infections
Internal Control (IC):
Indicator of perfect nucleic acid extraction
Monitoring PCR inhibition
To rule out failure of amplification

Cell Control (CC):
Quality of samples
Samples have been collected/transported/ stored properly

No Template Control (NTC):
To check for the absence of contamination

Positive Control (PC):
To monitor the whole process
CHOOSING THE RIGHT HPV TEST...

**COLLECTION MEDIUM**

- Conventional smear
- Liquid based cytology
  - Thinprep
  - Surepath
- SelfSampling
  - Evalynbrush
  - Qvintip
  - Colipee
  - ....
CHOOSING THE RIGHT HPV TEST...

**SENSITIVITY**

Analytical sensitivity

hrHPV infection
Must be high enough for:
- Epidemiology
- Status after treatment
- Status after vaccination

Clinical sensitivity

≥CIN2/3
(clinically relevant hrHPV infections)
Avoid false positives

**SPECIFICITY**

DNA vs RNA?

Key issue:
optimal balance between clinical sensitivity/specificity to minimize redundant or excessive follow-up procedures
In the case of HPV infections there is a big difference between analytical sensitivity and clinical sensitivity/specificity.

Characteristics of the ideal HPV Test:
- **High clinical sensitivity** ⇒ detect high-risk HPV infections that lead to cervical disease
  - High negative predictive value for disease (>99.5%)
- **High clinical specificity** ⇒ do not detect transient high-risk HPV infections that will not lead to cervical disease
  - High positive predictive value for disease

Is it possible to achieve 100% clinical sensitivity by increasing the analytical sensitivity?
- No, small gains in clinical sensitivity will result in a major decrease in clinical specificity (i.e. more false positives)
In order to become validated for use in cervical screening:
Candidate HPV assays should prove their value and assure high-quality and optimal safety
→ large prospective screening studies are time laborious

MEIJER criteria
• comparing with HC2 and GP5/GP6+
VALGENT
• Framework for HPV test comparison
• Verification of minimal criteria for use in cervical cancer screening
CHOOSING THE RIGHT HPV TEST...

**AUTOMATION/STANDARDISATION**

- High throughput / individual testing
- Batch/random access
- Chain of custody
- Starting from primary tube
- Minimalize hands on time
- Reduce contamination
A perfect HPV test should be a **clinically validated, robust, fully automated, fast, affordable, single assay** with an **adjustable cut-off** for detection, having a cell control and preferentially linked to a **genotyping** method.

**THE IDEAL METHOD DOES NOT EXIST!**

There is NO GOLDEN STANDARD
## Some commercial HPV tests

<table>
<thead>
<tr>
<th>Hybrid Capture 2 Qiagen</th>
<th>Abbott RealTime High Risk HPV Abbott</th>
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<tbody>
<tr>
<td>Cervista Hologic</td>
<td>Luminex HPV Genotyping,</td>
</tr>
<tr>
<td>Linear array Roche</td>
<td>Multimetrix/Progen</td>
</tr>
<tr>
<td>SPF10/LiPa DDL</td>
<td>Papillocheck, Greiner BioOne</td>
</tr>
<tr>
<td>Aptima Hologic</td>
<td>PreT ect HPV Proofer Norchip</td>
</tr>
<tr>
<td>Linear Array Extra HPV Genotyping Kit</td>
<td>Amplicor HPV Test Roche</td>
</tr>
<tr>
<td>Innogenetics</td>
<td>Xpert HPV Cepheid</td>
</tr>
<tr>
<td>Array Papillomavirus Genomica</td>
<td>BD Onclarity HPV Assay</td>
</tr>
<tr>
<td>Seeplex HPV Genotyping Seegene</td>
<td>...</td>
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<tr>
<td>HPV OncoTest Invirion Diagnostics</td>
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<tr>
<td>Cobas 4800 HPV test Roche</td>
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</table>

> 150 commercial available HPV tests
Methods for hpv detection

**Signal amplification**
- Increase a signal in proportion to the amount of DNA/RNA or protein target
- Initially not designed for genotyping
- Less sensitive

**Target amplification**
- Multiplicates fragments of DNA/RNA form a targeted sequence
- Use enzyme-mediated processes, to synthesize copies of target nucleic acid
- Produce $10^8$-$10^9$ copies of targeted sequences
- Sensitive to contamination, false-positive reaction
Methods for HPV detection

**Signal amplification**

- Southern Blot
- Northern Blot
- In situ hybridisation (ISH)
- Hybrid Capture
  - HC2 (Qiagen)
  - CARE HPV
- Invader technology
  - Cervista HPV (Hologic)
QIAGEN HYBRID CAPTURE ASSAY (HC2)

Clinically regarded as the “Gold Standard”
Signal amplification DNA screening assay
Targets 13 HR types (16 18 31 33 35 39 45 51 52 56 58 59 68)
hr and lr separation
Semi-automated system available
No internal control for sample integrity
Known issues regarding cross-reactivity
Hologic Cervista

Isothermal signal amplification DNA
Targets 14 HR types (16 18 31 33 35 39 45 51 52 56 58 59 66 68)
Separate test for high risk mixtures and 16/18 detection
Automated system
Contains cell control for sample sufficiency
Does not cross react with low risk types
Methods for hpv detection

Target amplification

→ PCR
  DNA amplification
  “consensus” primers in L1
  “type specific” primers in L1 or E6/E7
  Single vs multiplex format
  “Home Brew” vs Commercial

→ TMA
  Isothermal E6/E7 RNA amplification methods
Primers targeted to L1 gene

- Commonly used sets: PGMY09/11, GP5+/6+, SPF10
- → consensus primers having the ability to identify a large range of HPV types in one amplification
LIMITED TYPING

Type specific DNA/RNA sequence
Three HPV results in a single test

<table>
<thead>
<tr>
<th>Channel 1</th>
<th>Channel 2</th>
<th>Channel 3</th>
<th>Channel 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>18</td>
<td>31 33</td>
<td>CC</td>
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<td>18 45</td>
<td>35 39</td>
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<td>66 68</td>
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</table>
ABBOTT M2000 system

Automated batch assay
Target: L1 DNA

Limited typing:
- Individual result for 16 and 18
- Pooled results for 31 33 35 39 45 51 52 56 58 59 66 68

Cell control: β-globin
Clinical cut-off for CIN2+
Roche Cobas 4800

Highly automated batch assay
Target: L1 DNA
Limited typing:
  Individual result for 16 and 18
  Pooled results for 31 33 35 39 45 51 52 56 58 59 66 68
Cell control: β-globin
Clinical cut-off for CIN2+
HOLOGIC PANTHER

Highly automated random access assay
Target: E6/E7 mRNA
No typing / Limited typing:
  Individual result for 16 and 18/45
  Pooled results for 31 33 35 39 45 51 52 56 58 59 66 68
No Cell control

TomCat
Panther
Cepheid Genexpert

Fully integrated and automated single module on-demand system
One, two, four, 16, 48 or 80 module configuration
Rapid non batch test - POC
Cartridge

- Limited typing: 16 and 18/45 + others
- CC: HMBS
- Fast (+/- 1 hour)
- Expensive
- Cave: contamination
- Target: E6/E7 DNA
BD Onclarity HPV Assay

Highly automated batch assay
Target: E6/E7 DNA
Extended typing:
  Individual result for 16, 18, 31, 45, 51, 52
  3 pooled small groups (33, 58), (35, 39, 68) and (56, 59, 66)
Cell control: β-globin
“load and go” capability and ease of use

BD Viper LT System
FULL GENOTYPING

Reverse Line Blot assays (genotyping)
Type-specific oligoprobes

Roche Linear Array HPV Genotyping Test
- L1 consensus primer-based PCR with PGMY09/11 primers
- 37 different HPV types

INNO-LiPA HPV Genotyping
- SPF10 primers at the L1 region
- 24 low- and high-risk HPV types
Reverse hybridization assays (genotyping) Type-specific oligoprobes immobilized on:

**Microarrays**
- HPV oligo array (Kim et al. 2003)
- DNA array primer extension (APEX; Gheit et al. 2006)
- PapilloCheck (Greiner Bio-One)

**Microsphere beads**
- Multiplex HPV genotyping (MPG; Schmitt et al. 2006)
- HPV DNA suspension array (HPV-SA; Jiang et al. 2006)
- Templex assay (Han et al. 2006)
Bead-based multiplex genotyping
Disagreement between HPV assays

Figure 1. Primary samples, 30–65 years: Disagreement between Hybrid Capture 2, cobas, CLART, and APTIMA HPV assays. Proportions were calculated from all samples that tested positive on at least one assay. doi:10.1371/journal.pone.0086835.g001
HPV Testing Alone Misses Cervical Cancer

Landmark retrospective study of cervical cancer screening involving:

• Over 8.6 million women

• 256,648 biopsies

• 526 cases of cervical cancer

Women aged 30-65 were evaluated with both Pap+HPV testing. Histology results were paired to screening result to compare real-world performance of:

• Pap Alone

• HPV Alone

• Pap+HPV Together (Co-testing)

Evaluation of baseline screening results

• Biopsies taken within one year of initial screen

“It is important that the best and most sensitive diagnostic tools for cancer detection be identified and made available to all women. Our data support cotesting …as the most effective screening test for detecting cervical cancer.”

2. Quest Diagnostics press release April 14, 2015
Athena trial follow up study

The sensitivity for CIN3+ of cytology was 47.8% (95% CI; 41.6–54.1%) compared to 61.7% (95% CI; 56.0–67.5%) for the hybrid strategy (cytology if 25–29 years and cotesting with cytology and HPV if ≥30 years) and 76.1% (95% CI; 70.3–81.8%) for HPV primary (using Roche COBAS)
Conclusion

- Cervical cancer screening can be done using cytology, HPV testing or a combination of both
- Screening remains important even after vaccination
- Starting an HPV vaccination program without pre-vaccination knowledge of HPV prevalence or post-vaccination surveillance is unwise
  - Waiting for death statistics will take a long time
- HPV testing without counselling is causing stress on the system and the women
Conclusion of HPV testing

- A wide range of HPV detection and genotyping assays are available
- There is no golden standard for HPV testing
- Assays must be thoroughly validated to demonstrate actual clinical performance
- Assay variations and characteristics may change future testing algorithms
Clinicians and laboratorians must understand the clinical performance of the assays and critically evaluate and compare clinical trial data.

The choice for a method is dependent on its analytical and clinical sensitivities and specificities, and its purpose for use.

Molecular techniques for HPV detection and or genotyping still must become more rapid, automated, and low-cost to be of practical use in screening purposes.