HPV testing

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HPV detection

- HPVs can not be cultured in vitro
- No serological or protein 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)
  - Principle: hybridisation with complementary sequences

Target amplification: PCR

1st cycle 2nd cycle 3rd cycle 4th cycle

Some commercial HPV tests

- Hybrid Capture 2 Geno
- Digene HPV genotyping kit Roche
- Digene HPV genotyping kit Ld. Geno
- AmpliCyst HPV Test Roche
- Linear array Roche
- GC HPV InLUX 2XL
- Rodintha EasyQ HPV Biomark
- Astra Gen test
- Roehm Pankröck Viva reagents ThinPrep
- BIOCHIP DTS HPV LR Low
- Roche HPV GeneTarget HP Detection kit Geno/ID
- AID 300 test Geno/ID
- Progene HPV Typing kit Geno/ID
- AID HPV typing Geno/ID
- Linear ArrayViro / HPV Genotyping kit Inmegenest
- PDR Human Papillomavirus Detection kit Telara Meris Bio
- HPV Test EQ BV
- Array Papillomaviruses Garancie
- PrepTest HPV typing kit Biotech S.P.A
- PapTest Genova-Biolysera
- LIC HPV assay 3.5 Chiron
- Seegene HPV Genotyping Sargano
- Viroactiv Virofem
- HPV OneTest In Sure Diagnostics
- Genticis To HPV Real Time Quidel
- Roche RealTime High Risk HPV Assay
- Linear Array HPV genotyping Medac
- PampTest, Serona Bioltin
- ProTest HPV Printer Nacorbi

Test comparison on cervical scrapes of women with CIN3

N=45

Test comparison on cervical scrapes of women with normal cytology without high-grade CIN in follow-up

N=264
Principles most commonly used (hr)HPV DNA detection assays

- Hybridisation followed by signal amplification
  - Hybrid capture 2 (HC2; Qiagen): RNA probe cocktail (commercial)
  - Invader technology: Third Wave Invader HPV test (commercial)
- In situ hybridisation (ISH): in situ hybridisation of DNA with probe cocktail (commercial)
- Broad spectrum PCR: DNA amplification with consensus primers or multiplex format (some commercially available)

HrHPV E6/E7 mRNA detection assays

- Isothermal RNA amplification methods
  - PreTect HPV-Proofer (Norchip), NucliSense EasyQ HPV (Bomerieux): 5 hrHPV types
  - APTIMA (GenProbe): 13 hrHPV types

Commonly used HPV broad spectrum PCR assays target L1 sequences

Commonly used read-out systems for PCR assays

Enzyme immuno assays
- Cocktail of oligoprobes per well (no genotyping) (Eg. GP5+/6+-PCR, SPF10, Roche Amplicor)
- Reverse hybridization assays (genotyping); oligoprobes immobilized on:
  - Strips/filters
    - Linear Array (PGMY; Gravitt et al. 1998)
    - LIPA (SPF10; Kleter et al., 1999)
    - RLB (GP5+/6+-PCR; van de Brule et al., 2002)
    - Consensus HR HPV genotyping strip (GP5+/6+-PCR, PGMY, Amplicor; de Koning et al., 2006)

Example Enzyme Immuno Assay (EIA)

Enzyme immuno assay
Commonly used read-out systems for PCR assays

Enzyme immuno assays
- Cocktail of oligoprobes per well (no genotyping)
- Eg. GPS+6+PCR, SPF10, Roche Amplicor

Reverse hybridization assays (genotyping)
Type-specific oligoprobes immobilized on:
- Strips/filters
  - Linear Array (PGMY; Gravitt et al. 1998)
  - LIPA (SPF10; Keter et al., 1999)
  - RLB (GPS+6+PCR; van de Brule et al., 2002)
  - RH genotyping strip (GPS+6+PCR, PGMY, Amplicor; de Koning et al., 2000)

Reverse Line Blot (GP5+/6+ amplimers)
HPV types in samples

digene RH genotyping strip (Qiagen): genotyping of 18 HPV types (GP5+/6+ amplimers)

Commonly used read-out systems for PCR assays

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Novel assays: a.o. real-time multiplex PCR (eg. GenoID, Abbott)
HPV DNA tests can be used for several distinct purposes

- Epidemiological: assessing burden of HPV infections
- Vaccine monitoring: determining protection against incident HPV infections
- Cervical screening and triage: detecting/predicting disease
  - hrHPV testing should be considered for the detection of CIN2/3 or cancer, not simply viral infections

hrHPV testing for cervical screening and triage of women with Pap2/3a1

- Not necessarily hrHPV DNA presence per sé but elevated levels of viral DNA confer an increased risk of ≥CIN2/3 (Joseffson et al., Ylitalo et al., 2000)

Important distinctions

- Analytical sensitivity and specificity
  - ≥hrHPV infections
- Clinical sensitivity and specificity
  - ≥CIN2/3 (clinically relevant hrHPV infections)

hrHPV testing for cervical screening

- For screening hrHPV testing is not inherently useful unless it is considered in the context of CIN2/3 or cancer
- Key issue: optimal balance between clinical sensitivity/specificity
  - to minimize redundant or excessive follow-up procedures
- Current clinically validated tests (i.e. HC2 and GP5+/6+ PCR): clinical sensitivity of 90-95% for ≥CIN2/3
- Cautious against misguided attempts to achieve a 100% clinical sensitivity by increasing the analytical sensitivity
  - such small gains will result in a dramatic decrease in clinical specificity (i.e. more false positives)

Comparison study between GP5+/6+-PCR and SPF10-PCR

- 45 cases and 264 controls selected from women with normal cytology at baseline
- cases = women who developed lesions ≥CIN3
  - median age 33 years; median follow-up: 2.7 years
- controls = women with repeat normal cytology at follow-up or histology ≤CIN1
  - median age 41 years; median follow-up: 5.8 years

Hesselink et al., JCM 2008
### Nested case-control study women with normal cytology

**Cases:** CIN3  
**Controls:** ≤CIN1

<table>
<thead>
<tr>
<th></th>
<th>Overall</th>
<th>Screening cohort</th>
<th>Hospital cohort</th>
</tr>
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<tbody>
<tr>
<td>Cases</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Controls</td>
<td>%</td>
<td>%</td>
<td>%</td>
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<tr>
<td>N=25</td>
<td>p&lt;0.001</td>
<td>p=0.301</td>
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<tr>
<td>N=193</td>
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*SPF10 positivity rate was significantly higher than that of GP5+/6+-PCR for control women, not for case women.

### Viral load analysis in concordant vs discordant SPF10/GP5+/6+-PCR samples

**Type-specific real time PCR**

<table>
<thead>
<tr>
<th>HPV type</th>
<th>GP-/-SPF+ (n=13)</th>
<th>GP+/SPF+ (n=6)</th>
<th>GP+/SPF+ (n=4)</th>
<th>GP-/-SPF+ (n=14)</th>
<th>GP-/-SPF+ (n=15)</th>
<th>GP+/SPF+ (n=4)</th>
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<tr>
<td>HPV 16</td>
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<td>HPV 18</td>
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<tr>
<td>HPV 52</td>
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</tbody>
</table>

| log [HPV copies/scrape] | 9 | 8 | 7 | 6 | 5 | 4 | 3 |

**Comparison ISH (Ventana) and HC2**

- **Hospital population:** women referred to gynaecologist because of abnormal smear in screening program or post-treatment monitoring
- **LBC specimens (PreservCyt; n=76)**
- **Prescreened for positivity by hrHPV GP5+/6+-PCR**

**Clinical sensitivity of ISH too low: too many false negatives**

**HC2 ≈ GP5+/6+-PCR**

**ISH (Ventana) vs HC2**

- Clinical sensitivity of ISH too low: too many false negatives
- **HC2 = GP5+/6+-PCR**
ROC curve for HC2 values using cytology threshold >BMD

CONCLUSIONS

- hrHPV tests display variable clinical properties
- For cervical screening: high clinical sensitivity and at the same time high clinical specificity (limit false positives)
- At the moment only HC2 and GP5+/6+-PCR are known to fulfill this on the basis of data from large prospective trials
- Adherence of candidate tests to HPV test guidelines is necessary to avoid adverse affects of HPV testing

Proposal international guidelines for HPV test requirements for primary cervical screening (formulated relative to HC2)

Candidate test should:

1. Have a clinical sensitivity for ≥CIN2 not less than 90% of that of HC2 in women >30 years
   - Sensitivity HC2: 97.9% (95%CI: 95.9-99.9)
   - Translates into very high NPV (reassurance) allowing for extending screening intervals test neg. women
2. Have a clinical specificity for ≥CIN2 not less than 98% of that of HC2 in women >30 years
   - Specificity HC2: 91.3% (95%CI: 89.5-93.1)
3. Should display intra-laboratory reproducibility and inter-laboratory agreement with a lower confidence bound ≥87%

Validation guidelines of candidate HPV assays (comparative analysis with HC2)

1. Clinical sensitivity:
   - Compared to HC2, relative sensitivity of at least 90% assessed by non-inferiority score test
   - ≥60 smears of a representative set of women from a screening population with ≥CIN2 detected by HC2, either or not combined with cytology, and the candidate test (power of 80%)
   - When 2,500 samples tested: power >99%
2. Clinical specificity:
   - ≥800 smears of women ≥30 years without ≥CIN2 randomly taken from a screening population tested by HC2, either or not combined with cytology, and the candidate test (power of 80%)
3. Intra-laboratory reproducibility in time and inter-laboratory agreement:
   - ≥500 smears (incl. 30% positive in reference lab by a clinically validated test). Kappa at least 0.5

Laboratory requirements for HPV testing

1. Infrastructure for molecular testing in case nucleic acid amplification technology is used
2. Accreditation for clinical molecular testing
   - Standard operation procedures (SOP) and good laboratory practice
3. Quality control HPV test performance and sample processing
   - Proficiency testing including regular intra-laboratory evaluation
   - Inter-laboratory performance evaluated by sending proficiency panels

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Bead-based multiplex genotyping

HPV type-specific probes coupled to distinct coloured bead sets (one set per probe)
PCR
HPV genome

Luminex analyser

heat denaturation
hybridisation
biotinylated hybridised complexes

reporter dye

wash + analysis

wash

Schiessl et al., JCM 2005