The HORIZON Study
Different HPV tests, Different Horizons

Jesper Bonde
Molecular Pathology Laboratory
Dept. Pathology
Copenhagen University Hospital, Hvidovre
QIAGEN would like to thank our speaker, Dr. Jesper Bonde, for his presentation.

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Jesper Bonde,
PhD, Dipl.Med.Sci
Senior Researcher
Laboratory Manager
Molecular Pathology Laboratory
Dept. Pathology
Copenhagen University Hospital,
Hvidovre, Denmark

AxLab
BD Diagnostics
Genomica
Hologic/Gen-Probe
Qiagen
Roche Diagnostics
Roche Pharma
Today

Molecular HPV testing allows for improvements in cervical cancer prevention and screening, either as triage modality or as stand alone screening test

Tomorrow

The introduction of DNA and RNA based methods opens up a wide new horizon of methodologies to make screening individualized and highly specific for disease detection
Molecular HPV technology offers a new approach

The Premise – clinical perspective

All cervical cancers caused by HR-HPV infection, active or latent over a period of years

High sensitivity CIN2+ in the range of >90% favours HPV assays over cytology (Very variable from 55%-80%)

Of current cervical screening technologies, molecular HPV testing offers by far the best negative predictive value for detection of high grade CIN (Dillner et al. BMJ, 2008)

A negative test result provides long term confidence that disease is not imminent

Vaccination will reduce disease making cytology even more challenged
**Molecular HPV technology offers a new approach**

*The Premise – Laboratory perspective*

Molecular HPV testing is more reproducible than cytology

Molecular HPV testing can be automated and QA/QC controlled

Not prone to intra-observer variability as cytology

National and international QA/QC programs will eventually allow for performance evaluation in order to secure uniform services

Negative predictive value of HPV tests means longer, safe intervals reducing the cost of screening allowing a re-focusing on those women who really need follow up and/or treatment
What is The HORIZON Study
Randomised control trials

Shows that HPV testing is superior to cytology in detecting CIN2+/CIN3+, or other endpoint

Referral population split-sample trials

Compares clinical performance of various HPV assays w/wo concordant cytology

Assay comparisons/cell validation

Shows that an assays performs clinically and analytically according to e.g. Meijer Guidelines (2009)

Population based screening trials

Evaluates different HPV assays in real life settings
The HORIZON study is an independent investigator driven study

The company partners accepted and acknowledged the protocol prior to the study

All instrumentation and software were manufacturer issued, and maintained for the duration of the study in both studies
Context
The National Danish screening Program
Cervical cancer screening programs started in Denmark in late 1960’s

Current coverage is 76%

By screening early stages can be diagnosed

When early stages are treated, invasive disease can be avoided

It worked

However, 20% of all women with cervical cancer had a recent normal cytology (Ingemann-Hansen et al, BJC, 2008)
The current screening in Denmark

**Population**: 1,560,000 women nationwide (23-65 years)

**Who is offered screening?**
- All women 23-49 years: Every 3rd year
- All women ≥50-65: Every 5th year

**"Smears"**
- Denmark: Approx. 450,000/annually
- Hvidovre Hospital: Approx. 150,000/annually

**Type of collection media**
- All “smears” are LBC, less than 5% pap’s.
- LBC is 80% SurePath, 20% Thinprep

**Automated reading**
- Focal point computer assisted screening is widely used

Consolidation of screening units into large units improves consistency and service
Current indications for HPV tests by the Danish National Board of Health, 2012

Triage (ASCUS ≥30 years)

1st control after conisation (LEEP)

Primary screening ≥60 years (Check out test, DNA only, starting from 2013)

Annual turnover of HPV tests:

75,000 National/25,000 at Hvidovre Hospital from 2013

Equals:
1 of 6 LBC samples in our lab will have an HPV test by full implementation of these indications
What do we need from Molecular HPV screening tests?

“In the fight to reduce a rare disease, cervical cancer, let’s not introduce a common disease, hr-HPV.”

**TRIAGE**
- High Clinical Specificity
- Good sensitivity

**EXIT TEST**
- High sensitivity
- Sample sufficiency control

**TEST of CURE**
- High sensitivity
- Genotyping?

**PRIMARY SCREENING**
- High Clinical Specificity
- Good sensitivity
The HORIZON Design
Comparing APTIMA, CLART, cobas & HC2 HPV assays in PRIMARY cervical cancer screening

Clinical and analytical assay performance in a true screening study sample

Evaluation parameters
Intra-laboratory assay reproducibility on screening samples

Evaluation of assay specific cross reactivity profiles to LR HPV genotypes

SurePath and HPV assays

Cross comparison of assays in primary screening of women ≥30 years
Four HPV assays – four different technologies

- **Cobas® HPV test**  Real-time PCR assay, with co-detection of HPV HR and 16 and 18

- **HC2®**  Hybridization assay with HR HPV detection

- **APTIMA®**  RNA assay with HR HPV detection

- **CLART®**  PCR-Microarray assay with simultaneous detection of 35 genotypes

The HORIZON Study
Different HPV tests, Different Horizons
Design & Inclusion

12,138 consecutive, unselected samples received at Dept. between 10th June, 2011 – 25th August, 2011

6,258 samples

Residual material added 2ml SurePath

N=5,064

LBC

HC2 (Post quot)

DILUTION 1:1 in SurePath

N=1,194

Discarded

LBC and HC2 only

Aptima

Cobas

CLART

N=5,064

N=1,194

Discarded

LBC and HC2 only
Outcomes & Follow-up

Cytology positives: Referral for follow-up according to National guidelines

Cytology negative/HPV positive on any test:
- Clinical trial follow-up at 18 months
- Re-test on all 4 tests

Cytology negative/HPV negative on any test:
- Return to screening program
Baseline

AIM:
- Functionality of different HPV assays
- Baseline prevalence using different assays
- Reproducibility, cross reactivity
- Bench marking
- Assay-by-assay comparison

Follow-up

AIM:
- Sensitivity & specificity for detecting severe abnormalities (≥CIN3)
- + psychosocial effects of testing positive
- + cost-effectiveness analysis

Figure from Nature Biotechnology Reviews
The Horizon Study

Baseline data, prevalence & genotypes
Baseline data from the HORIZON study

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Routine samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤22</td>
<td>3.2% (162)</td>
</tr>
<tr>
<td>23-29</td>
<td>30.3% (1,534)</td>
</tr>
<tr>
<td>30-65</td>
<td>64.3% (3,256)</td>
</tr>
<tr>
<td>≥66</td>
<td>2.2% (112)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cytology</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ASCUS</td>
<td>7.3% (371)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HPV assays</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cobas</td>
<td>26.8% (1,356)</td>
</tr>
<tr>
<td>CLART</td>
<td>25.1% (1,273)</td>
</tr>
<tr>
<td>HC2</td>
<td>20.4% (1,035)</td>
</tr>
<tr>
<td>APTIMA</td>
<td>16.7% (846)</td>
</tr>
</tbody>
</table>

[Rebolj et al., forthcoming]
Baseline data from the HORIZON study

Genotype distribution in all 5064 unselected samples

Bonde et al., forthcoming
Goldman et al., Vaccine 2013
Assay Performance Indicators

The importance of laboratory performance

Intra-laboratory assay Reproducibility
Implications for primary screening

Importance of intra-laboratory reproducibility

Need to trust your system
In primary screening each sample will run only once
Need to know the limitations of the systems to design National QA & QC procedures & guidelines

Negative reproducibility:
Important for the safety of extended screening intervals for HPV negative

Positive reproducibility:
Important for frequency of false positive tests

Overall reproducibility:
Important for lab performance
Overall assay reproducibility with respect to prevalence findings

Preisler et al., PLOS One, 2013
Bonde et al., forthcoming
Rebolj et al., J Mol. Diag, 2013
The importance of laboratory performance

Cross reactivity to low risk genotypes
Effect on current screening

**Definition:**
A cross reacting sample is defined as a HPV HR positive result that only contains LR-genotypes by full genotyping reference.

**Analytical cross reactivity evaluation against plasmid:**
Is it representative?

**Effect on primary screening:**
In primary screening cross reactivity lowers the specificity of the HPV screening, cause false positives, and result in too many follow ups and referrals, lowering efficiency of the programme.

**Does cross reactivity matter?**
In daily practice it matters for patient safety, QA and QC.
## Overall cross reactivity

<table>
<thead>
<tr>
<th></th>
<th>N\textsubscript{Total} = 5,064</th>
<th>cobas® (DNA L1)</th>
<th>HC2® (DNA)</th>
<th>Aptima® (RNA E6/E7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total # HPV positive findings</td>
<td>26.7% (1,356)</td>
<td>20.4% (1,034)</td>
<td>16.7% (846)</td>
<td></td>
</tr>
<tr>
<td>Total # HPV LR only</td>
<td>49</td>
<td>50</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Absolute Cross-Reactivity</td>
<td>0.97%</td>
<td>0.99%</td>
<td>0.55%</td>
<td></td>
</tr>
<tr>
<td>Relative Cross-Reactivity</td>
<td>3.6%</td>
<td>4.8%</td>
<td>3.3%</td>
<td></td>
</tr>
</tbody>
</table>

[Bonde et al., forthcoming]
The HORIZON Study is the first study evaluating four HPV assays in a routine laboratory in charge of a population-based screening program.

The four assays had an overall comparable assay reproducibility, 93-98%.

Cross reactivity to LR was below 1% for APTIMA, cobas, and HC2 as compared with CLART.

SurePath is a suitable media for HPV testing, with only one sample out of 5,064 returning an invalid result.
The HORIZON Study
Study results from the epidemiology perspective
Baseline data from the HORIZON study

Preisler et al., PLOS ONE, 2013
Bonde et al., forthcoming
Rebolj et al., J.Mol.Med 2013
Goldman et al., Vaccine 2013
Horizon: The surprising variability in assay outcomes

### Assay

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## Agreement of the four HPV assays

<table>
<thead>
<tr>
<th></th>
<th>All 5,064 samples</th>
<th>23-29 years</th>
<th>30-65 years</th>
<th>30-65 years</th>
<th>30-65 years, primary screening samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>23-29 years</td>
<td>30-65 years</td>
<td>30-65 years</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>N (% )</td>
<td>N</td>
<td>N (%)</td>
</tr>
<tr>
<td>≥1 test pos</td>
<td>1,636</td>
<td>1,534</td>
<td>3,256</td>
<td>2,881</td>
<td>375</td>
</tr>
<tr>
<td></td>
<td>(100%)</td>
<td>(100%)</td>
<td>(100%)</td>
<td>(100%)</td>
<td>(100%)</td>
</tr>
<tr>
<td>1 test pos</td>
<td>28%</td>
<td>18%</td>
<td>38%</td>
<td>40%</td>
<td>30%</td>
</tr>
<tr>
<td></td>
<td>(40%)</td>
<td>(19%)</td>
<td>(34%)</td>
<td>(38%)</td>
<td></td>
</tr>
<tr>
<td>2 test pos</td>
<td>20%</td>
<td>21%</td>
<td>20%</td>
<td>21%</td>
<td>19%</td>
</tr>
<tr>
<td></td>
<td>(13%)</td>
<td>(22%)</td>
<td>(13%)</td>
<td>(19%)</td>
<td></td>
</tr>
<tr>
<td>3 test pos</td>
<td>52%</td>
<td>61%</td>
<td>41%</td>
<td>39%</td>
<td>50%</td>
</tr>
<tr>
<td></td>
<td>(41%)</td>
<td>(73%)</td>
<td>(50%)</td>
<td>(73%)</td>
<td></td>
</tr>
</tbody>
</table>

[Rebolj et al., submitted]
Agreement of the four HPV assays: Primary screening, 30-65 years

Sum of all proportions: 100% (all women testing positive on at least one HPV assay)

Only 29% of all HPV+ samples tested positive on all 4 assays

All combinations of positive tests for the four assays were observed in the data

[Rebolj et al., submitted]
Is this an odd finding, from an odd country, with odd data?
Primary screening, 30-65 years

Pairwise agreement of the four HPV assays:

Concordance for Linear Array, Cervista, Abbott & GP5+/6+

Gage et al., HC2 vs LA: 50%; Poljak et al., HC2 vs. Abbott: 67%; Carrozzi et al., HC2 vs. Abbott: 60%; Kurian et al., HC2 vs. Cervista: 49%; Quigley et al., HC2 vs. Cervista: 54%; Meijer et al., HC2 vs. GP5+/6+: 56%

[Nielsen et al., in preparation]

[Rebolj et al., submitted]
And this means:

Concordance in HPV assay positivity is Age, screening history, cytology dependent

- Poorer in women ≥30 years
- Poorer in screening samples
- Poorer in women with normal cytology
What are the clinical consequences of these findings?
Colposcopy referral: Triage by cytology

Sum of all proportions: 100% (all women testing positive on at least one HPV assay having abnormal cytology)

Only 68% of all HPV+/cyt+ women would be referred to colposcopy regardless of which of the four assays was used

[Rebolj et al., in preparation]
Results of repeated testing in 18 months

Women aged 30-65 years with HPV+/cyt- samples in primary screening:

<table>
<thead>
<tr>
<th>Screening test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytology</td>
<td>9% became cytology abnormal → colposcopy</td>
</tr>
<tr>
<td>Hybrid Capture 2</td>
<td>47% remained positive on HC2 → colposcopy</td>
</tr>
<tr>
<td>cobas</td>
<td>49% remained positive on cobas → colposcopy</td>
</tr>
<tr>
<td>CLART</td>
<td>37% remained positive on CLART → colposcopy</td>
</tr>
<tr>
<td>APTIMA</td>
<td>37% remained positive on APTIMA → colposcopy</td>
</tr>
</tbody>
</table>

[Rebolj et al., in preparation]
Detection of ≥CIN 2 (preliminary)

Sum of all proportions: 100% (all ≥CIN 2)

83% of all ≥CIN 2 were detected with any of the four assays

The remaining 17% of all ≥CIN 2 showed relatively good concordance: they were often detected by three assays

[Rebolj et al., in preparation]
Detection of ≥CIN 3 (preliminary)

Very similar as ≥CIN 2

CLART (N=36)  COBAS (N=36)  APTIMA (N=33)

HC2 (N=34)

5 %  5 %  4 %

81 %  3 %  3 %

[Rebolj et al., in preparation]
Agreement of the four HPV assays:
Primary screening, 30-65 years

Sum of all proportions: 100% (all women testing positive on at least one HPV assay)

Only 29% of all HPV+ samples tested positive on all 4 assays

All combinations of positive tests for the four assays were observed in the data

[Rebolj et al., submitted]
3 women had cervical cancer

<table>
<thead>
<tr>
<th>Cytology</th>
<th>HC2 result (rlu/co)</th>
<th>Cobas result (CT)</th>
<th>CLART result (genotypes)</th>
<th>APTIMA (s/co)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSIL</td>
<td>Positive (11.8)</td>
<td>Positive, HPV 16 (28.6)</td>
<td>Positive (HPV 16)</td>
<td>Positive (13.90)</td>
</tr>
<tr>
<td>HSIL</td>
<td>Positive (21.43)</td>
<td>Positive, HPV 18 (39.5)</td>
<td>Negative</td>
<td>Positive (0.84)</td>
</tr>
<tr>
<td>HSIL</td>
<td>Positive (92.02)</td>
<td>Negative*</td>
<td>Positive (HPV 16)</td>
<td>Positive (11.10)</td>
</tr>
</tbody>
</table>

* CT-β: 28.9

[Rebolj et al., in preparation]
More studies on primary screening with new assays are needed
Consider your needs, consider the evidence.

**TRIAGE**
- High Clinical Specificity
- Good sensitivity

**EXIT TEST**
- High sensitivity
- Sample sufficiency control

**TEST of CURE**
- High sensitivity
- Genotyping?

**PRIMARY SCREENING**
- High Clinical Specificity
- Good sensitivity
Another perspective of HPV screening

Self-sampling
Why self sampling

The definitively most efficient way to improve screening efficacy is to *raise* attendance rate

Self sampling offers itself to molecular HPV testing, but can not be done using cytology

The aim of self sampling is to get more women to go for a regular screening test
Evaluating use of two different sampling devices
Lavage versus dry swap

HPV testing performed using both HC2 and Genomica CLART genotyping in a split sample design

To be followed by targeted self sample trial with 12-25,000 invited non-attendees in Capital Region, Copenhagen, 2014-2015
Norwegian Cancer Register Pilot 2013

Women eligible for 2nd remainder letter are randomized within age groups (1600)*per month in the Oslo region

27-34 years (600)*
Reference group 300
Screening as usual
Delphi Screener (150)
Evalyn Brush (150)

Test 300
Women are sent a letter with information about upcoming study with the option to decline

34-49 years (600)*
Reference group 300
Screening as usual
Delphi Screener (150)
Evalyn Brush (150)

Test 300
Women are sent a letter with information about upcoming study with the option to decline

50-69 years (400)*
Reference group 200
Screening as usual
Delphi Screener (100)
Evalyn Brush (100)

Test 200
Women are sent a letter with information about upcoming study with the option to decline

Based on numbers from the Cancer Registry of Norway 2012, Reference 3.
<table>
<thead>
<tr>
<th>Age groups</th>
<th>Control group</th>
<th>Test group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Attended screening after remainder letter</td>
<td>Used self-sampling device</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>%</td>
</tr>
<tr>
<td>26-34</td>
<td>848</td>
<td>14,9</td>
</tr>
<tr>
<td>35-49</td>
<td>981</td>
<td>15,3</td>
</tr>
<tr>
<td>50-69</td>
<td>764</td>
<td>13,7</td>
</tr>
<tr>
<td>Total</td>
<td>2593</td>
<td>14,7</td>
</tr>
</tbody>
</table>
# Norwegian Cancer Register Pilot 2013

<table>
<thead>
<tr>
<th>Age groups</th>
<th>N</th>
<th>Positive(s)</th>
<th>%</th>
<th>Positives</th>
<th>%</th>
<th>Positives</th>
<th>%</th>
<th>Positives</th>
<th>%</th>
<th>Positives</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>26-34</td>
<td>59</td>
<td>20</td>
<td>33,9</td>
<td>13</td>
<td>22,0</td>
<td>19</td>
<td>32,2</td>
<td>12</td>
<td>20,3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evalyn</td>
<td>31</td>
<td>11</td>
<td>35,5</td>
<td>8</td>
<td>25,8</td>
<td>10</td>
<td>32,3</td>
<td>7</td>
<td>22,6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delphi</td>
<td>28</td>
<td>9</td>
<td>32,1</td>
<td>5</td>
<td>17,9</td>
<td>9</td>
<td>32,1</td>
<td>5</td>
<td>17,9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35-49</td>
<td>52</td>
<td>10</td>
<td>19,2</td>
<td>8</td>
<td>15,4</td>
<td>7</td>
<td>13,5</td>
<td>5</td>
<td>9,6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evalyn</td>
<td>28</td>
<td>7</td>
<td>25,0</td>
<td>5</td>
<td>17,9</td>
<td>5</td>
<td>17,9</td>
<td>3</td>
<td>10,7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delphi</td>
<td>24</td>
<td>3</td>
<td>12,5</td>
<td>3</td>
<td>12,5</td>
<td>2</td>
<td>8,3</td>
<td>2</td>
<td>8,3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50-69</td>
<td>47</td>
<td>6</td>
<td>12,8</td>
<td>5</td>
<td>10,6</td>
<td>4</td>
<td>8,5</td>
<td>3</td>
<td>6,4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evalyn</td>
<td>24</td>
<td>2</td>
<td>8,3</td>
<td>2</td>
<td>8,3</td>
<td>1</td>
<td>4,2</td>
<td>1</td>
<td>4,2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delphi</td>
<td>23</td>
<td>4</td>
<td>17,4</td>
<td>3</td>
<td>13,0</td>
<td>3</td>
<td>13,0</td>
<td>2</td>
<td>8,7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total*</td>
<td>158</td>
<td>36</td>
<td>22,8</td>
<td>26</td>
<td>16,5</td>
<td>30</td>
<td>19,0</td>
<td>20</td>
<td>12,7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evalyn</td>
<td>83</td>
<td>19</td>
<td>22,9</td>
<td>15</td>
<td>18,1</td>
<td>14</td>
<td>16,9</td>
<td>10</td>
<td>12,0</td>
<td></td>
<td></td>
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<tr>
<td>Delphi</td>
<td>74</td>
<td>16</td>
<td>21,6</td>
<td>11</td>
<td>14,9</td>
<td>12</td>
<td>16,2</td>
<td>7</td>
<td>9,5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Baseline data from the HORIZON study

Genotype distribution in all 5064 unselected samples

Self sampling Data

HPV16: 12%
Single infections: 61%
Multiple infections: 38%
In summary
The challenge of HORIZON

Choose assay by the intended application; be that primary screening, triage, check-out testing, or ToC. Choose HPV tests that are validated... also against the sampling media you use.

More than one assay might be the right solution for your screening program, targeting different applications. This is the challenge of HPV screening. The HORIZON Study.
The HORIZON Group
a multi-disciplinary team

Centre for Epidemiology & Screening, University of Copenhagen
Elsebeth Lynge
Matejka Rebolj

Dept. Pathology & Clinical Research Centre, Copenhagen University Hospital, Hvidovre
Carsten Rygaard
Sarah Preisler
Ditte Ejegod
Jette Junge
Jesper Bonde

Sponsor
Danish Strategic Research Council

Corporate Partners
Genomica
Hologic/Gen-Probe
Qiagen
Roche
Questions?

Jesper.Hansen.Bonde@regionh.dk