New Horizons in Cervical Cancer Screening

Combining molecular and cytology based technologies to provide a solution for future of cervical screening

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Hologic/Gen-Probe  
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Roche Pharma
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All instrumentation and software were manufacturer issued, and maintained for the duration of the study in both studies
From cytology to primary HPV screening
Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials

*Figure 2: Cumulative detection of invasive cervical carcinoma
*Observations are censored 2.5 years after CIN2 or CIN3 detection, if any.

Ronco et al, Lancet Onc. 2014
Primary HPV screening has a higher sensitivity than cytology for disease detection, allows for a high degree of automation, and is highly reproducible.

The downside is a higher number of screening false positives compared to cytology.

“In the fight to reduce a rare disease, cervical cancer, let’s not introduce a common disease, hr-HPV.”
Current work-flow for primary screening
European guidelines recommend cytology as triage, September 2015

If negative, return to screening
Interval: 5-7 years

If positive, triage and follow-up

What do we need from a molecular HPV test?
Screening false-positive samples remain the challenge for primary HPV screening
The Evolution of HPV tests

- **2003**: Digene – QIAGEN Hybrid Capture II
  - 13 HR Pool
  - No genotyping

- **2009**: Third Wave – Hologic Cervista™ HPV and HPV 16/18 genotyping
  - 14 HR Pool + Reflex 16, 18
  - Limited genotyping

- **2011**: Roche cobas® 4800 HPV and HPV 16/18 genotyping
  - HPV 16/18 + 12 HR Pool

- **2011/2012**: GenProbe – Hologic Aptima HPV and HPV 16, 18+45 genotyping
  - 14 HR Pool + Reflex 16, 18+45

- **2014**: Abbott HPV Primary Screening
  - HPV 16/18 + 12 HR Pool

- **2015**: BD Onclarity™ HPV 16, 18, 45 31, 51, 52, 33_58, 35_39_68, 56_59_66
  - Extended genotyping

*Arbyn et al., J Clin Virol. 2016*
**Predictive Value of HPV Genotype**

*The HPV genotype matters!*

Absolute Risk of CIN3+ in 33,288 Danish Women with Normal Cytology, follow up data 2015

HPV 31 / 33 has the same / greater 3-5 year longitudinal risk as HPV 18 in Danish Women

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Risk by Genotypes may be regional

**Copenhagen, Denmark**
Highest relative risk;
HPV 18, 16, 31, 33

**Milano, Italy**
Highest relative risk;
HPV 16, 33/58, 31

*Sandri MT et al, Forthcoming*
Current work-flow for primary screening
HPV incl. Risk based assessment by genotyping & cytology triage

- HPV+ or – w./genotyping embedded in the screening test design

HPV screening test → Cytology Triage → Follow-up

If negative, return to screening
Interval: 5-7 years

If positive, triage and follow-up
Choice of LBC and technology defines the performance of the screening program
Challenge:
Primary HPV screening will rely on LBC to triage the HPV positive women to reduce false positive rates and over-treatment

Question:
Which LBC system provides the best detection of abnormalities?
**Method:**
We looked at the performance of LBC over a *15 years period (1995-2007)* with various degrees of assisted reading and compared it to the performance of conventional Pap. All screening samples included a follow up period of *2.5 years*.

Using data from five nationwide registers, technological phases were identified by slide preparation, reading technique, and triage of borderline cytology.

**Measurement indicators:**
Trends in the detection of *cervical intraepithelial neoplasia (CIN)* were an indicator of the technology’s relative sensitivity, and trends in false-positive tests an indicator of relative specificity.
From conventional Pap’s to liquid based cytology (LBC)

- **SurePath Technology**, N=394,056 samples
  - Implemented 2002

- **ThinPrep Technology**, N=151,135 samples
  - Implemented 2004

- **Conventional Pap**, N=129,057 samples
  - From 1998
Definition of the technology phases

- **1998**
  - All labs use Conventional

- **2013- Today**
  - SurePath LBC, BD FocalPoint Computer-assisted Slide Profiler, PrepStain
    - Profiler 50% cut off
    - Profiler 25% cut off
      - HPV triage of ASCUS
      - Focal point Imaging system
    - HPV triage of ASCUS
    - ThinPrep LBC
      - Manual read
      - T3000 Slide processor
      - Image system, HPV triage

By Phase 2, shift to LBC
<table>
<thead>
<tr>
<th></th>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Phase 3</th>
<th>Phase 4</th>
<th>Phase 5</th>
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</thead>
<tbody>
<tr>
<td><strong>Conventional</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASCUS</td>
<td>2%</td>
<td>3%</td>
<td>2%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CIN2</td>
<td>0.22%</td>
<td>0.21%</td>
<td>0.18%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>≥CIN3</td>
<td>0.52%</td>
<td>0.58%</td>
<td>0.57%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>SurePath</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASCUS</td>
<td>4%</td>
<td>4%</td>
<td>5%</td>
<td>6%</td>
<td>7%</td>
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<tr>
<td>CIN2</td>
<td>0.37%</td>
<td>0.34%</td>
<td>0.45%</td>
<td>0.56%</td>
<td>0.68%</td>
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<tr>
<td>≥CIN3</td>
<td>1%</td>
<td>0.92%</td>
<td>1.21%</td>
<td>1.49%</td>
<td>1.65%</td>
</tr>
<tr>
<td><strong>ThinPrep</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASCUS</td>
<td>5%</td>
<td>3%</td>
<td>3%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CIN2</td>
<td>0.25%</td>
<td>0.15%</td>
<td>0.32%</td>
<td>-</td>
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<tr>
<td>≥CIN3</td>
<td>0.75%</td>
<td>0.78%</td>
<td>0.74%</td>
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</table>
SurePath and FocalPoint technology detected significantly more ≥CIN3 disease, than conventional Pap.

ThinPrep did not significantly detect more ≥CIN3 disease than conventional Pap.
SurePath and FocalPoint technology detected significantly more ≥CIN3 disease than conventional Pap. The higher sensitivity for ≥CIN2 lead to a significant decrease in specificity.

ThinPrep imaging system implementation lead to a significant decrease in PPV for ≥CIN3
Cervical histology after routine ThinPrep or SurePath liquid-based cytology and computer-assisted reading in Denmark

Matilde Rødby1,2, Johanne Rask3, Marjooin van Ballegooijen4, Benny Kristensen5, Kristen Rozenblatt6, Jesper Bindslev-Jensen7, Carsten Rigtved8 and Elisabeth Lyng9

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Background: We compared the sensibility and specificity of liquid-based cytology (LBC) and computer-assisted reading for SurePath/FocalPoint and ThinPrep with those of manually read conventional cytology in routine cervical screening in four Danish laboratories.

Methods: Data from five national Cervical Cancer Screening Program registries were included. Cervical smears were scored using the Bethesda System. The following diagnostic criteria were used: CIN1, CIN2, and CIN3 were defined as mild, moderate, and severe cervical intraepithelial neoplasia, respectively. The study group consisted of women aged 16 years or older. A total of 9,249 samples were included in the study.

Results: The sensitivity of LBC was significantly higher than that of conventional cytology (p < 0.05). The specificity of LBC was also higher than that of conventional cytology (p < 0.05). The area under the ROC curve for LBC was 0.85 (95% CI 0.82-0.87), while that for conventional cytology was 0.78 (95% CI 0.75-0.80). The positive predictive value (PPV) of LBC was 0.89 (95% CI 0.87-0.91), while that of conventional cytology was 0.82 (95% CI 0.80-0.84). The negative predictive value (NPV) of LBC was 0.94 (95% CI 0.93-0.95), while that of conventional cytology was 0.90 (95% CI 0.89-0.91).

Conclusions: LBC is a superior method for the diagnosis of cervical cancer, with higher sensitivity and specificity than conventional cytology. The area under the ROC curve for LBC was significantly higher than that for conventional cytology (p < 0.05). The PPV and NPV of LBC were also significantly higher than those of conventional cytology (p < 0.05).

At 23–29 years:
- **SurePath/FocalPoint** statistically significantly increased the detection of ≥CIN3 by 85% compared with manually read conventional cytology.
- The 11% increase with **ThinPrep** was not significant.

At 30–44 years:
- Increased detection with **SurePath/FocalPoint** was 58%;
- 16% increase with **ThinPrep** was not significant.

At 45-59 years: No differences
So my claim is that I will go with the LBC technology that detects the highest ≥CIN2 in the pre-HPV world to ensure that I detect the most relevant cases amongst the HPV positive in HPV primary screening.
Internationally validated HPV tests
Meijer validated commercial HPV assays

<table>
<thead>
<tr>
<th>HPV test</th>
<th>Reference test used</th>
<th>Sample medium</th>
<th>Author and publication year</th>
</tr>
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<tbody>
<tr>
<td>GP5+/GP6+</td>
<td>HC2</td>
<td>ThinPrep</td>
<td>Meijer et al., 2009</td>
</tr>
<tr>
<td>Greiner Bio-one PapilloCheck assay</td>
<td>GP5+/GP6+</td>
<td>ThinPrep</td>
<td>Hesselink et al., 2010</td>
</tr>
<tr>
<td>Roche cobas 4800 HPV test</td>
<td>HC2</td>
<td>ThinPrep</td>
<td>Heideman et al., 2011</td>
</tr>
<tr>
<td>Abbott Real Time HR HPV Assay</td>
<td>GP5+/GP6+</td>
<td>ThinPrep</td>
<td>Hesselink et al., 2013</td>
</tr>
<tr>
<td>Hologic Aptima HPV assay</td>
<td>GP5+/GP6+</td>
<td>ThinPrep</td>
<td>Heideman et al., 2013</td>
</tr>
<tr>
<td>BD Onclarity HPV assay</td>
<td>HC2</td>
<td>ThinPrep</td>
<td>Ejegod et al., 2013</td>
</tr>
<tr>
<td>Hologic Cervista</td>
<td>HC2</td>
<td>ThinPrep</td>
<td>Boer et al., 2014</td>
</tr>
</tbody>
</table>

So far no HPV commercial test has been validated using SurePath screening samples
• The clinical specificity for a new HPV test has to be $\geq 0.98$ of the clinical specificity of the reference test (Standard: $\geq 800$ women with $<$CIN2 histological follow-up)

• The clinical sensitivity for a new HPV test has to be $\geq 0.90$ of the clinical sensitivity of the reference assay. (standard: $\geq 60$ women with histological confirmed $\geq$CIN2)

• Lower confidence bound for intra and inter laboratory reproducibility has to be 87% in a population of $\geq 500$ sample where a least 30% are HPV positive
Control group for specificity calculation

1154 women from primary screening without ≥CIN2
Follow-up
Standard: ≥800 women

Clinical results

2840 Collected from the routine

1189 Excluded due too little volume for testing

1651

381 Excluded due to missing HC2 and/or Onclarity test

1270

1154 Included in the study

116 Excluded due to follow-up history

As reference test we used HC2 (Qiagen)

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1 Data registration agency AHH-2015-087/04154
2 Ethical committee protocol J. no. H-4-2012-070
Case group for sensitivity calculation

61 women with ≥ASCUS cytology and ≥CIN2 histological Follow-up\(^2\)
Standard: ≥60 women

411
Samples from women with ≥ASCUS

10
Excluded due too little volume for testing or invalid HPV result

401

125
Excluded due lack of ≥CIN2 follow-up history

276
Samples from women with ≥CIN2 follow up

61
Samples from women ≥30 years with ≥CIN2 follow up

125
Excluded due lack of ≥CIN2 follow-up history

10
Excluded due too little volume for testing or invalid HPV result

61
Samples from women ≥30 years with ≥CIN2 follow up

10
Excluded due too little volume for testing or invalid HPV result

215
Excluded from women <30 year

1 Data registration agency AHH-2015-087/04154
2 Ethical committee protocol J. no. H-4-2012-070
Clinical results

Specificity for <CIN2

- Onclarity: 1034/1154 (0.90)
- HC2: 1038/1154 (0.90)
- Relative specificity: 1.0 (0.97-1.02)

Sensitivity for ≥CIN2

- Onclarity: 59/61 (0.97)
- HC2: 60/61 (0.98)
- Relative sensitivity: 0.98 (0.93-1.04)

1154 women from primary screening without ≥CIN2
Follow-up¹
Standard: ≥800 women

61 women with ≥ASCUS cytology and ≥CIN2
histological Follow-up²
Standard: ≥60 women

Calculation of validation status based on Non-inferiority test:

Clinical specificity: p=0.02, Onclarity meets criteria
Clinical sensitivity: p=0.02, Onclarity meets criteria

¹ Data registration agency AHH-2015-087/04154
² Ethical committee protocol J. no. H-4-2012-070
**Assay reproducibility**

**Intra laboratory reproducibility**
(Samples tested x 2 in CPH lab)

<table>
<thead>
<tr>
<th>Copenhagen 2</th>
<th>POS</th>
<th>NEG</th>
<th>Total</th>
</tr>
</thead>
<tbody>
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<tr>
<td>NEG</td>
<td>10</td>
<td>357</td>
<td>367</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td><strong>500</strong></td>
</tr>
</tbody>
</table>

Overall reproducibility: 97.4%
Positive reproducibility: 92.8%
Lower confidence bound: 95.9%

**Inter-laboratory reproducibility**
(Samples tested in CPH lab and in Milano lab)

<table>
<thead>
<tr>
<th>Milano</th>
<th>Copenhagen 1</th>
<th>POS</th>
<th>NEG</th>
<th>Total</th>
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<tbody>
<tr>
<td>POS</td>
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<td>9</td>
<td>142</td>
<td></td>
</tr>
<tr>
<td>NEG</td>
<td>7</td>
<td>351</td>
<td>358</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
<td><strong>500</strong></td>
</tr>
</tbody>
</table>

Overall reproducibility: 96.8%
Positive reproducibility: 95%
Lower confidence bound: 95.2%

*Standard*: >87%
• **BD Onclarity HPV assay meets the Meijer Guidelines for**
  – Clinical specificity ✓
  – Clinical sensitivity ✓
  – Intra- and inter laboratory reproducibility ✓

*The BD Onclarity HPV Assay on Samples Collected in SurePath Medium Meets the International Guidelines for Human Papillomavirus Test Requirements for Cervical Screening*  

• **Meijer validation also obtained on ThinPrep samples**

  (Ejegod et al., 2013)
In conclusion:

The BD Onclarity HPV assay is validated on SurePath collected samples according to the International guidelines.

The BD Onclarity HPV assay is the only HPV test with international validation for use with both ThinPrep and SurePath collected screening samples.
HPV Primary screening is the best choice

Cytology’s new role will be to facilitate the best triage of HPV HR positive samples

Implementation of self sampling and HPV testing will improve the screening program if implemented efficiently
Acknowledgement

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&
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Elsebeth Lynge
Thank you for your attention