A Comparison of the Performance of Different HPV Tests in ThinPrep vs SurePath

PREDICTORS 4

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630 women referred for colposcopy

Two cervical brush samples taken by clinicians

Randomised

Sample 1 – (S)
brush head left in 10ml SurePath solution

Sample 2 – (T)
brush agitated in 20ml ThinPrep PreservCyt solution

Randomised

0.5ml aliquot for HC2 (Qiagen) and 4 ml in ThinPrep
0.7 ml aliquot for RealTime (Abbott)
0.5ml aliquot for Onclarity (BD)
0.5ml aliquot for Aptima (Hologic)
0.5ml aliquot for OncoHealth
0.5ml aliquot for Paptype (Genera Biosystems)

Comparison of positivity/ viral load
## Assay cut-off

<table>
<thead>
<tr>
<th>Assay</th>
<th>Assay HPV types</th>
<th>Measurement</th>
<th>Positive if value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC2 (Qiagen)</td>
<td>Nucleic acid amplification test – 13 HR types</td>
<td>RLU/CO value</td>
<td>≥ 1</td>
</tr>
<tr>
<td>RealTime (Abbott)</td>
<td>PCR HPV detection (14 HR types) &amp; partial genotyping (16 &amp; 18)</td>
<td>Ct value</td>
<td>≤ 32</td>
</tr>
<tr>
<td>Onclarity (BD)</td>
<td>PCR for detection of 9 HR HPV types/groups</td>
<td>Ct value</td>
<td>≤ 34.2</td>
</tr>
<tr>
<td>Aptima (Hologic)</td>
<td>E6/E7 mRNA – 14 HR types</td>
<td>S/CO value</td>
<td>≥ 0.5</td>
</tr>
<tr>
<td>OncoHealth</td>
<td>E6/E7 oncoprotein detection using ELISA</td>
<td>Mean OD/CO value</td>
<td>≥ 1</td>
</tr>
<tr>
<td>Paptype (Genera Biosystems)</td>
<td>Full HPV genotyping – 14 HR types</td>
<td>Signal/CO value</td>
<td>&gt;0.0004 for HPV58</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt;0.0003 for HPV68</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt;0.0002 for all other types</td>
</tr>
</tbody>
</table>
# Worst histology

<table>
<thead>
<tr>
<th>Histology (biopsy/LLETZ result)</th>
<th>Frequency</th>
<th>% of total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No disease</strong></td>
<td></td>
<td>53</td>
</tr>
<tr>
<td>No histology (within 9 months of baseline)</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td>Inadequate sample</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>243</td>
<td></td>
</tr>
<tr>
<td><strong>Low grade disease</strong></td>
<td></td>
<td>19</td>
</tr>
<tr>
<td>HPV only</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>CIN1</td>
<td>116</td>
<td></td>
</tr>
<tr>
<td><strong>High-grade disease including squamous cell cancer</strong></td>
<td></td>
<td>27</td>
</tr>
<tr>
<td>CIN2</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>CIN3</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>Invasive squamous cell carcinoma</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Glandular disease including adenocarcinoma</strong></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>CGIN high-grade (+/- CIN)</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Invasive Adenocarcinoma</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>630</td>
<td>100</td>
</tr>
</tbody>
</table>
## Correlations between two collection media
### Signal Strength measure

<table>
<thead>
<tr>
<th>Test</th>
<th>N</th>
<th>Spearman’s $\rho$ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC2 (Qiagen)</td>
<td>344</td>
<td>0.87 (0.84, 0.89)</td>
</tr>
<tr>
<td>Onclarity (BD) – All</td>
<td>630</td>
<td>0.94 (0.93, 0.95)</td>
</tr>
<tr>
<td>RealTime (Abbott) – All</td>
<td>630</td>
<td>0.87 (0.85, 0.89)</td>
</tr>
<tr>
<td>Paptype (Genera) – All</td>
<td>585</td>
<td>0.85 (0.82, 0.87)</td>
</tr>
<tr>
<td>APTIMA (Hologic)</td>
<td>613</td>
<td>0.82 (0.79, 0.84)</td>
</tr>
<tr>
<td>OncoHealth</td>
<td>630</td>
<td>0.17 (0.10, 0.25)</td>
</tr>
</tbody>
</table>
# Concordant and Discordant Pairs

**ThinPrep (T) vs SurePath (S)**

<table>
<thead>
<tr>
<th>Test</th>
<th>N</th>
<th>T+ (%)</th>
<th>S+ (%)</th>
<th>Discordant pairs</th>
<th>Observed agreement (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC2 (Qiagen)</td>
<td>344</td>
<td>84</td>
<td>78</td>
<td>28 / 8</td>
<td>90</td>
</tr>
<tr>
<td>Onclarity (BD) All</td>
<td>630</td>
<td>77</td>
<td>78</td>
<td>5 / 13</td>
<td>97</td>
</tr>
<tr>
<td>RealTime (Abbott) All</td>
<td>630</td>
<td>76</td>
<td>71</td>
<td>34 / 5</td>
<td>94</td>
</tr>
<tr>
<td>Paptype (Genera) All</td>
<td>585</td>
<td>79</td>
<td>80</td>
<td>17 / 21</td>
<td>94</td>
</tr>
<tr>
<td>APTIMA (Gen-Probe)</td>
<td>613</td>
<td>78</td>
<td>73</td>
<td>45 / 15</td>
<td>90</td>
</tr>
<tr>
<td>OncoHealth</td>
<td>630</td>
<td>57</td>
<td>48</td>
<td>168 / 113</td>
<td>55</td>
</tr>
<tr>
<td>Onclarity (BD) 16</td>
<td>630</td>
<td>25</td>
<td>25</td>
<td>2 / 6</td>
<td>99</td>
</tr>
<tr>
<td>RealTime (Abbott) 16</td>
<td>630</td>
<td>25</td>
<td>24</td>
<td>8 / 3</td>
<td>98</td>
</tr>
<tr>
<td>Paptype (Genera) 16</td>
<td>585</td>
<td>27</td>
<td>27</td>
<td>10 / 9</td>
<td>97</td>
</tr>
<tr>
<td>Onclarity (BD) 18</td>
<td>630</td>
<td>9</td>
<td>9</td>
<td>1 / 2</td>
<td>99</td>
</tr>
<tr>
<td>RealTime (Abbott) 18</td>
<td>630</td>
<td>9</td>
<td>8</td>
<td>3 / 1</td>
<td>99</td>
</tr>
<tr>
<td>Paptype (Genera) 18</td>
<td>585</td>
<td>13</td>
<td>14</td>
<td>9 / 14</td>
<td>96</td>
</tr>
</tbody>
</table>
Diagnostic Accuracy CIN2+ for ThinPrep

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC2</td>
<td>0.967</td>
<td>0.206</td>
</tr>
<tr>
<td>Abbott</td>
<td>0.949</td>
<td>0.319</td>
</tr>
<tr>
<td>BD</td>
<td>0.972</td>
<td>0.306</td>
</tr>
<tr>
<td>Genera</td>
<td>0.934</td>
<td>0.261</td>
</tr>
<tr>
<td>Aptima</td>
<td>0.977</td>
<td>0.304</td>
</tr>
<tr>
<td>OncoHealth</td>
<td>0.597</td>
<td>0.447</td>
</tr>
</tbody>
</table>
Diagnostic Accuracy CIN2+ for SurePath

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC2</td>
<td>0.956</td>
<td>0.281</td>
</tr>
<tr>
<td>Abbott</td>
<td>0.915</td>
<td>0.37</td>
</tr>
<tr>
<td>BD</td>
<td>0.972</td>
<td>0.289</td>
</tr>
<tr>
<td>Genera</td>
<td>0.94</td>
<td>0.254</td>
</tr>
<tr>
<td>Aptima</td>
<td>0.931</td>
<td>0.354</td>
</tr>
<tr>
<td>OncoHealth</td>
<td>0.523</td>
<td>0.54</td>
</tr>
</tbody>
</table>
Diagnostic Accuracy CIN3+ for ThinPrep

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC2</td>
<td>0.978</td>
<td>0.181</td>
</tr>
<tr>
<td>Abbott</td>
<td>0.99</td>
<td>0.287</td>
</tr>
<tr>
<td>BD</td>
<td>1</td>
<td>0.27</td>
</tr>
<tr>
<td>Genera</td>
<td>0.956</td>
<td>0.235</td>
</tr>
<tr>
<td>Aptima</td>
<td>1</td>
<td>0.264</td>
</tr>
<tr>
<td>OncoHealth</td>
<td>0.583</td>
<td>0.438</td>
</tr>
</tbody>
</table>
Diagnostic Accuracy CIN3+ for SurePath

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC2</td>
<td>0.978</td>
<td>0.247</td>
</tr>
<tr>
<td>Abbott</td>
<td>0.969</td>
<td>0.337</td>
</tr>
<tr>
<td>BD</td>
<td>1</td>
<td>0.255</td>
</tr>
<tr>
<td>Genera</td>
<td>0.956</td>
<td>0.227</td>
</tr>
<tr>
<td>Aptima</td>
<td>0.989</td>
<td>0.32</td>
</tr>
<tr>
<td>OncoHealth</td>
<td>0.552</td>
<td>0.536</td>
</tr>
</tbody>
</table>
Diagnostic Accuracy: CIN2+ for ThinPrep & SurePath

- PreservCyt
- HC2
- Abbott
- BD
- Genera
- Aptima
- OncoHealth

- SurePath
- HC2
- Abbott
- BD
- Genera
- Aptima
- OncoHealth

Sensitivity

- 0.80
- 0.85
- 0.90
- 0.95
- 1.00

Specificity

- 0.45
- 0.40
- 0.35
- 0.30
- 0.25
- 0.20
- 0.15
Diagnostic Accuracy: CIN3+ for ThinPrep & SurePath

- PreservCyt
- HC2
- Abbott
- BD
- Genera
- Aptima
- OncoHealth

Sensitivity

Specificity

SurePath

- HC2
- Abbott
- BD
- Genera
- Aptima
- OncoHealth

0.80
0.85
0.90
0.95
1.00

0.45
0.4
0.35
0.3
0.25
0.2
0.15
log(1+SurePath) = -0.046 + 0.816 \times \log(1+\text{ThinPrep})

ThinPrep vs. SurePath
(N = 344)

ThinPrep HC2: RLU/CO values
SurePath HC2: RLU/CO values
BD Onclarity

ThinPrep vs. SurePath

SurePath = 0.049 + 0.976 x ThinPrep
Abbott – Real Time
ThinPrep vs. SurePath

SurePath = 6.860 + 0.818 x ThinPrep
Genera – full typing

ThinPrep vs. SurePath

SurePath = 0.193 + 0.855 x ThinPrep

SurePath Genera: log(1+Signal values/CO)
ThinPrep Genera: log(1+Signal values/CO)
Hologic - APTIMA

ThinPrep vs. SurePath

SurePath = 0.140 + 0.807 x ThinPrep
Conclusions

With the exception of OncoHealth:

• Good observed agreement between ThinPrep and SurePath for discordant and concordant pairs (range 89 to 99%)
• Very strong correlation of signal strength between the two collection media - ThinPrep versus SurePath (range 0.82 to 0.94)
• Sensitivities were in excess of 90% for CIN2+ and 95% for CIN3+ for both media and with no significant differences except for a lower sensitivity for CIN2+ of Aptima in SurePath (93% vs 98%, P = 0.005).
• Viral loads typically lower with SurePath (except BD)
  • Specificity generally better with SurePath
• Statistically significant differences between SurePath as Sample 1 versus SurePath as Sample 2 for all tests