An E6 Oncoprotein Based Diagnostic Test for Cervical Pre-Cancer and Cancer

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INTRODUCTION:
In developing countries, cervical cancer is a leading cause of cancer-related death of women due to the lack of implementation of appropriate screening tests for cervical pre-cancer and cancer. A screening test for low-resource settings should be simple, rapid, cost-effective, and sensitive and specific for detection of lesions needing clinical intervention.

Arbor Vita Corp. (AVC), in collaboration with PATH, has developed a rapid diagnostic test, the AV Avantage HPV E6 Test, that detects E6 oncoprotein from cervical swabs. E6 (in concert with E7) is necessary for cervical cell transformation to occur. Consequently, an E6 oncoprotein-based diagnostic test bears the promise to be especially specific for detection of those pre-cancerous lesions that have progressed to a high-grade CIN stage or to cancer (Figure 1).

The AV Avantage HPV E6 Test uses high affinity mAb for the specific capture and detection of high-risk HPV-E6 oncoproteins in a lateral flow based format. The test does not require complex equipment, nor does it require a cold chain for transport and storage. The current prototype can detect and type E6 protein of HPV types 16, 18, and 45 (Table 1, Figure 2). A version that is still under development will detect E6 oncoprotein of the “top 7” prevalent HPV types (HPV 16, 18, 45, 31, 33, 52, 58), accounting for ~90% of cervical cancers.

RESULTS
Performance in a Clinical Pilot Study:
To examine, whether detection of E6 oncoprotein was feasible from cervical specimens and was more clinically specific than HPV DNA, we conducted a small clinical pilot study. Cervical swab samples of women with confirmed pathology (normal, CIN1, CIN3, CIN3+, and cancer) were tested on the AV Avantage HPV E6 Test (Figure 3A). HPV typing was performed via PCR linear array. Of 91 specimens that were positive for HPV16 and/or HPV18 and/or HPV45, 16 specimens were pathology-negative or CIN1, 34 specimens were CIN3, 25 specimens were CIN3+, and 16 specimens were cancers. None of the negative or CIN1 specimens tested positive in the AV Avantage HPV E6 Test, but 20 (59%) of the CIN3, 17 (68%) of the CIN3+, and 4 (88%) of the cancers tested positive. (Figure 3B). Negative or positive test outcomes could easily be interpreted by visual inspection (Figure 3C).

CONCLUSIONS:
- The E6 oncoprotein-based AV Avantage HPV E6 Test does not require complex equipment or a cold chain for storage.
- In a small clinical pilot study, the test detected high-grade lesions and cancers, but not CIN1 or pathology negative specimens.
- The HPV 16/18/45 test will enter a large clinical study in China (START-UP) in Q3 of 2010.

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Arbor Vita Corp. maintains a collaboration with Drs. G. Orfanoudakis, G. Trave and E. Weiss of the University Louis Pasteur of Strasbourg. One of the anti-E6 mAbs used for this work was kindly provided by Dr. E. Weiss (Giovanna C., et al., J. Mol. Recog. 1999;12:141-152).
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Table 1

<table>
<thead>
<tr>
<th>HPV16/18/45 Test Positive</th>
<th>Pathology</th>
<th>DNA</th>
<th>E6</th>
<th>%[E6/DNA]</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>8</td>
<td>0</td>
<td>0%</td>
<td>0-37%</td>
<td></td>
</tr>
<tr>
<td>CIN1</td>
<td>5</td>
<td>0</td>
<td>0%</td>
<td>0-37%</td>
<td></td>
</tr>
<tr>
<td>CIN3</td>
<td>34</td>
<td>20</td>
<td>60%</td>
<td>41-75%</td>
<td></td>
</tr>
<tr>
<td>CIN3+</td>
<td>25</td>
<td>17</td>
<td>68%</td>
<td>46-85%</td>
<td></td>
</tr>
<tr>
<td>CxCa</td>
<td>16</td>
<td>14</td>
<td>88%</td>
<td>62-98%</td>
<td></td>
</tr>
<tr>
<td>≤CIN1</td>
<td>16</td>
<td>0</td>
<td>0%</td>
<td>0-37%</td>
<td></td>
</tr>
<tr>
<td>CIN3+</td>
<td>16</td>
<td>0</td>
<td>0%</td>
<td>0-37%</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>91</td>
<td>51</td>
<td>56%</td>
<td>45-66%</td>
<td></td>
</tr>
</tbody>
</table>

* For part of the specimens, distinction between CIN3 and cancer was not provided.
Those specimens are designated “CIN3+”.

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