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 HPV 18 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 14 (88%) of the cancers tested positive. (Figure 3B). Negative or positive test outcomes could easily be interpreted by visual inspection (Figure 3C).

CONCLUSIONS

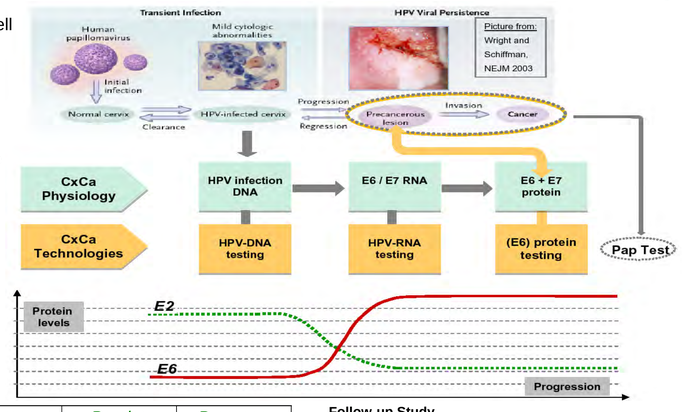
- **The E6 oncoprotein-based AV Avantage HPV E6 Test does not require complex equipment or a cold chain for storage.**
- **In a small clinical pilot study, the test detected high-grade lesions and cancers, but not CIN1 or pathology negative specimens.**
- **The HPV 16/18/45 test will enter a large clinical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

**** Organized by PATH, NIH, and the Chinese Academy of Sciences (CICAMS).**

Figure 1

E6 Oncoprotein as a Biomarker of HPV Viral Activity

- Cervical epithelial cell transformation requires E6 and E7 oncoproteins.
- E6 oncoprotein levels may correlate with severity of CIN lesions and risk for progression.



	Regress	Persist	Progress
HPV-16, 18 or 45-E6 Protein Positive	1	0	1 (endpoint: CIN3)
HPV-E6 Protein Negative	20	6	1 (endpoint: CIN2)

Follow-up Study

6,557 Chinese women, previously unscreened for CxCa were recruited for participation in START. Women with histologically confirmed CIN1 were entered into the cohort of the CIN1 Follow Up Study (n=179). All women (n=142) were screened again after 2 years. Women who tested HPV positive by RNA testing (n=29) were tested for E6 expression.

• *Data originally presented by Sellors, J.W. et al. at the 2007 IPV Conference in Beijing, presentation 1C-07.*

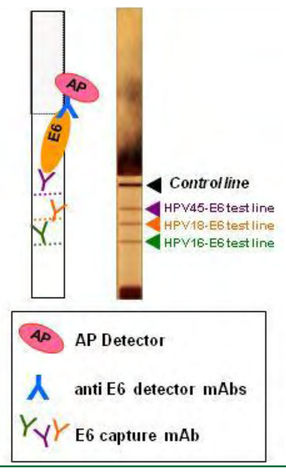
Table 1

AV Avantage HPV E6 Test

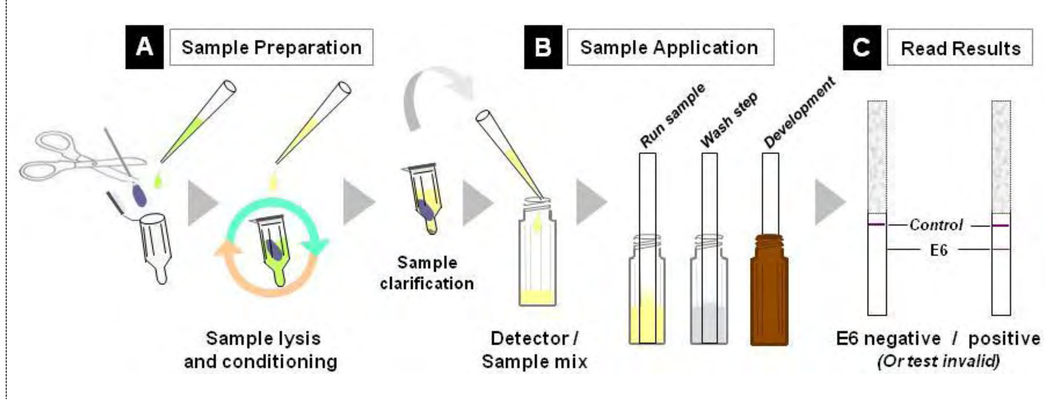
- **Point of care test**
- **Sample type: cervical swabs**
- **Simple, inexpensive, robust, requires cold chain**
- **Requires no complex equipment**
- **Marker: HPV-E6 protein of HPV types 16/18/45**
  (Development towards detection of additional types ongoing)
- **Target sensitivity: < 50 pg E6 per cervical swab**
  (corresponds to ~ 50,000 CxCa cells or 5 pg per test strip; 1/10 of total specimen is loaded per strip)

Figure 2

The Principle….



The Work Flow….



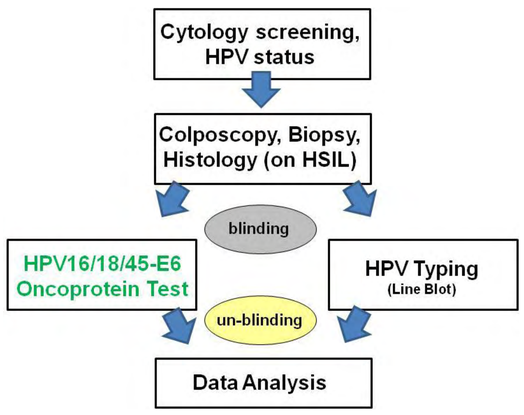
Time from sample collection to result: ~ 120 min.

3-Strip Test Unit

Figure 3

Results from a Small Clinical Pilot Study

A) Study Setup

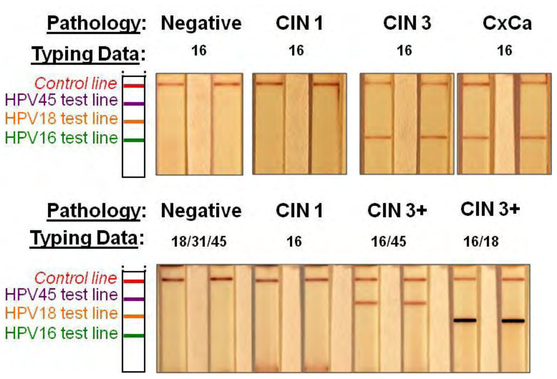


B) E6 Oncoprotein in Clinical Specimens

	HPV16/18/45 Test Positive			
Pathology	DNA	E6	%[E6/DNA]	95% CI
Negative	8	0	0%	0-37%
CIN1	8	0	0%	0-37%
CIN3	34	20	59%	41-75%
CIN3+*	25	17	68%	46-85%
CxCa	16	14	88%	62-98%
≤CIN1	16	0	0%	0-37%
CIN3+	75	51	68%	56-78%
Total	91	51	56%	45-66%

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

C) Representative Test Results



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