

A Test For Detection of HPV-16 E6

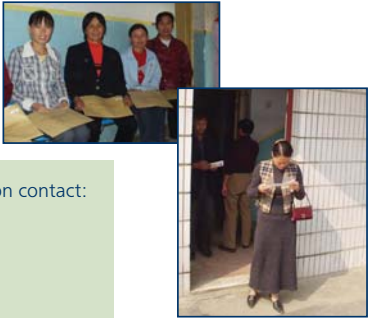
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Introduction

Cervical cancer is a disease which kills 230,000 women annually. Low-resource regions of the world are disproportionately burdened with 80% of the cases. High-risk human papillomavirus (HPV) types have been identified as the etiological agent for over 99% of cervical cancers. Infection with HPV is ubiquitous and often is resolved by the host. However, infections resulting in cervical cancer require the expression of high-risk HPV E6 and E7 oncoproteins beginning at some point during the transformation from precancerous lesions into cancer. An assay for the detection of high-risk HPV E6 may help to accurately identify women at increased risk of progression to cancer.

PDZs are a class of conserved protein domains involved in numerous cellular protein-protein interactions. They have widespread biological functions including cell-to-cell contact, intercellular signaling, and cell polarity. PDZ ligands are C-terminal sequences following the general motif: **X-S/T-X-V/L**

Regulatory proteins of many viral proteins exhibit PDZ ligand C-terminal sequences that can bind to cellular PDZs. E6 proteins of only high-risk HPV types, not of the low-risk types, interact with PDZs. Arbor Vita Corporation and PATH have collaborated on the development of an assay based on the specific interaction of high-risk HPV E6 with PDZ protein and detection via an anti-HPV E6 mAb. Such a sandwich ELISA has been developed and is now being adapted to an immunochromatographic strip (ICS) platform for use in low-resource settings.



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Methods

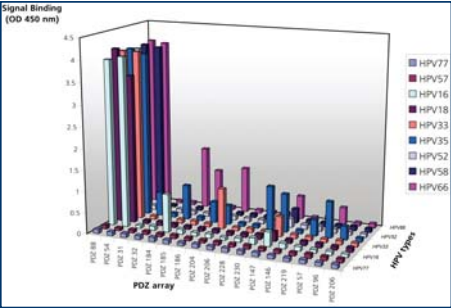
The complete set of known PDZ proteins was tested against high-risk HPV E6. The PDZ protein that exhibited the greatest binding strength with high-risk HPV E6 was selected as the capture reagent.

The recognition of PDZs to only high-risk HPV E6 provides a high degree of selectivity for the assay.

Novel antibodies to HPV E6 from high-risk HPV types were generated. The antibodies were screened for affinity and cross-reactivity with other HPV E6. The antibody with the strongest reactivity to HPV-16 E6 was selected as the detector.

Magnetic ICS was selected for evaluation as the assay platform based on the ease of use and acceptability of ICS tests in low-resource settings, inherent sensitivity of the technology, and ability to quantitate results.

Selection of the “PDZ Oncogenic E6 Detector”



Binding of seven high-risk and two low-risk HPV E6 C-terminal peptides to the Arbor Vita “human-PDZ domain reference set” was tested via ELISA. Representative binding data for 17 different PDZs are shown. Only peptides representing high-risk E6, but not those representing low-risk E6, showed binding to PDZ domains. PDZ88 bound all oncogenic E6 tested.

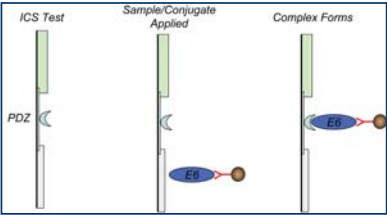
Procedure

The PDZ capture reagent was immobilized on nitrocellulose membrane.

Assays were assembled with a laminate backing, membrane with immobilized PDZ, hydrophilic sample pad, absorbent pad, and membrane cover tape. Test strips were cut to 7.5 mm wide.

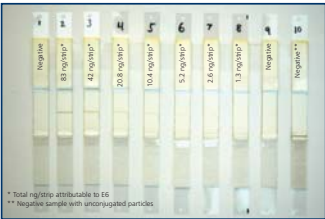
The detector antibody was covalently conjugated to superparamagnetic particles.

Doubling dilutions of recombinant HPV-16 E6 from 83 ng/assay to 1.3 ng/assay were prepared in running buffer. The detection reagent was mixed with the sample and applied to the strip. Upon migration of the sample, the strips were inserted into the magnetic strip reader. The strip reader scanned the strip membrane and generated data curves. The peak height was selected and data were reported as relative magnetic units (RMU).

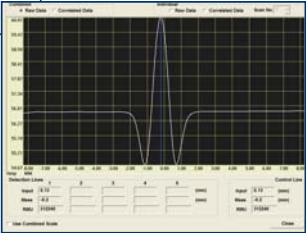
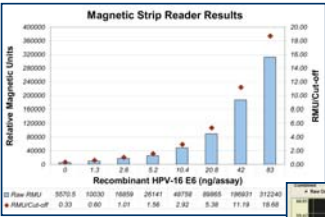


The positive/negative cut-off was set at three times the average of the negative controls. The RMU for each sample was divided by the cut-off. Samples greater than 1 were considered positive while samples less than 1 were considered negative.

Data



RMU Data	
1	5116
2	312240
3	186931
4	89865
5	48758
6	26141
7	16859
8	10030
9	6025
10	281



Conclusions

The current magnetic ICS assay is capable of detecting recombinant HPV-16 E6 down to 2.6 ng/assay.

E6 expression in cervical cancer cells has been determined to be approximately 1 ng/10⁶ cells. The ELISA has been shown to detect oncogenic E6 from cancer cell lines equivalent to 30,000 cells.

Next steps include further optimization to increase sensitivity and evaluating cell cultures and clinical specimens. Once utility with HPV16 is demonstrated, additional high-risk HPV types will be added to the assay.