Developing a Lateral Flow Strip Test for E6 Oncoprotein to Identify Cervical Intraepithelial Neoplasia (CIN) at Increased Risk of Progression to Cervical Cancer

As part of the START project, ArborVita Corporation (AVC) and PATH are collaborating to optimize test methods for an affordable, rapid, point-of-care test for the detection of high-risk human papillomavirus (HPV) E6 and to determine the limit of detection for each method.

**Objective**

Cervical cancer kills 230,000 women annually. Low-resource regions of the world are disproportionately burdened with 80% of the cases. Efficient screening methods are the key to decreasing the death toll from this disease. High-risk HPV types have been identified as the etiological agent for 99% of cervical cancers. Infection with HPV is ubiquitous and often is resolved by the host. High-risk HPV infections leading to cervical cancer require the production of both HPV E6 and E7 oncoproteins. An assay capable of detecting high-risk HPV E6 from cervical swab samples may have a high positive predictive value and may help to accurately identify women at increased risk of progression to cancer.

As part of the START Project, AVC and PATH are collaborating to develop such a test in a rapid immunochromatographic strip (ICS) format that will be suitable for use in low-resource settings. The assay is based on AVC’s proprietary technology which utilizes the specific interaction of high-risk HPV E6 with PDZ protein and detection via an anti-HPV E6 antibody.

**Principles**

PDZs (named for the first three described domain-containing proteins—postsinptic density 95, Discs, large, and zona occludens) are a conserved class of protein domains that engage in protein-protein interactions by binding proteins at a short C-terminal sequence known as a PDZ ligand (PL). Cellular proteins exhibiting PDZ domains fulfill widespread biological functions, including cell-to-cell contact, intercellular signaling, and cell polarity. Numerous viruses encode proteins that have PLs, thus allowing the viral protein to interact with the cellular PDZ domain-containing proteins. E6 proteins of high-risk HPV E6 but not low-risk HPV E6 have demonstrated high affinity with one PDZ. The PDZ-E6 binding provides a high level of selectivity for the assay.

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

**Methods**

Two ICS platforms are being evaluated for the best limit of detection: one which uses an antibody colloidal gold conjugate which is visually interpreted yielding qualitative results, and the other which uses an antibody paramagnetic particle conjugate which is read by an instrument yielding quantitative results (MAR assay, Development System, MagnalBioSciences, San Diego, CA, USA).

**Procedures**

- **Magnetic ICS tests** were characterized with serial dilutions of a recombinant E6 protein representative of high-risk HPV-16 in the assay buffer. Negative samples consisted of assay buffer alone or an excess of maltose binding protein, the recombinant protein tag.
- **Gold ICS tests** were characterized with serial dilutions of recombinant HPV-16 protein in lysate from cervical cell swabs from HPV-negative patients with the assay buffer. HPV-16 E6 stable expressing cell lines were generated using the HPV-negative cancer cell line C33A. Lysates of the negative and HPV-16 E6 positive cell lines were prepared with the assay buffer. Serial dilutions of the lysates were evaluated with the gold ICS tests.
- Sample was applied to the ICS test and migrated across the reaction zone via capillary flow.
- At 20 minutes the magnetic ICS tests were read on the strip reader yielding relative magnetic units (RMUs). The RMUs for the samples were related to the cut-off based on the reactivity of the negative controls to determine the limit of detection.
- At 30 minutes the gold-based ICS tests were visually interpreted. The presence of a line at the reaction zone indicated a positive, while the absence of such a line was interpreted as a negative.

**ICS Test Principles**

A. PDZ is immobilized as the reaction zone of the immunochromatographic strip. B. The sample containing E6 and antibody conjugated detector are applied to the sample pad. The sample tracts to regions on the strip by capillary flow. C. As the sample passes the reaction zone, Au/Ag colloidal gold-based latex particles are visualized while the paramagnetic particle-based strips are detected with a magnetic strip reader.

**ICS Test Procedure**

1. Collect cervical swab sample. 2. Add buffer to sample. 3. Let strip sit for 10 minutes. 4. Add strip to sample. 5. Read strip. 6. Compare to control. 7. Results.

**Magnetic ICS Data**

- **Recombinant HPV-45 E6 Limit of Detection**
  - Average RMU ± StDev (Range)
  - 0 pg: 34 ± 6 (10 to 64) 152 0.20 (0.002 to 0.79)
  - 1750 pg: 24688 ± 5833 (20776 to 34604) 24.9 12.50 (9.76 to 16-26)
  - 750 pg: 10685 ± 8445 (9890 to 114900) 7.40 5.02 (4.64 to 5.54)
  - 350 pg: 6999 ± 3670 (3844 to 95148) 24.2 3.34 (2.33 to 5.40)
  - 253 pg: 36062 ± 6165 (29186 to 44278) 16.9 1.72 (1.17 to 2.80)
  - 175 pg: 32086 ± 8074 (24531 to 40679) 24.8 1.53 (1.13 to 1.91)

**Gold ICS Data**

- **Limit of Detection**

**Conclusions**

- The magnetic ICS platform has demonstrated a limit of detection ~175 pg E6 based on testing with recombinant protein.
- The gold-based ICS platform has demonstrated a limit of detection ~160 pg E6 based on testing with recombinant protein. Testing with lysates from cells producing high-risk HPV E6 indicate a limit of detection of 90,000 to 155,000 cells.
- Internal studies have shown that cervical cancer cell lines produce ~1 ng E6/1,000,000 cells. Work is continuing to quantify E6 in cervical cell samples from clients with cancer, CIN 2/3, and CIN 1. Protein determinations on provider-collected cervical swabs indicate that the swabs collect between 500,000 and 2,000,000 cells. Based on these findings, the target limit of detection for an E6 assay is ~50 pg.
- Next steps include further assay optimization and evaluation of signal amplification mechanisms to achieve the required limit of detection.

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