Novel Flow Cytometry-based Assay for Detection of E6/E7 mRNA in Cervical Specimens

Michael Mira

Translational Diagnostics, Enzo Biochem Inc.
Why E6/E7?

In 10-15% of patients, a persistent HPV infection will develop.

*These patients are at the greatest risk for developing invasive cancer!*

Over-expression of E6 and E7 oncoproteins is a critical step toward HPV-related disease progression and cancer.

Challenges around existing E6 and E7 assays:

- Inability to analyze expression at the single cell level
- Complexity of the method and lack of proper controls
- Low specificity which negatively impacts patient care

How does the assay work?

1. Fixation
   - Cell Suspension
   - ThinPrep or SurePath

2. Permeabilization

3. Hybridization of probes with target sequences
   - Unbound probes will not fluoresce in absence of target sequence

4. Analysis of thousands of cells in one test by using the flow cytometer

Small sample volume required
   Multiple tests can be run from one specimen

Homogeneous assay design eliminates the need for extensive sample washing prior to data acquisition

Enzo
Innovation Through Pioneering Technology™
Representative NILM Sample

Representative HSIL Sample

Selecting for ectocervical cells by cell morphology

Measuring E6 E7 expression by specific labeled probes
### Assay Precision Near the Limit of Detection

#### Positive Control Cell Dilutions

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<tr>
<th>Dilution Level</th>
<th>% POS</th>
<th>% NEG</th>
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![Graph showing positive cell dilutions](image)

- **R² = 0.9997**

*Enzo*

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## Assay Precision Near the Limit of Detection

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Mean = 1.45%  Mean = 0.92%  Mean = 0.45%

SD = 0.26%  SD = 0.20%  SD = 0.18%
FlowScript™ HPV E6/E7 Assay Features

• Homogeneous system omits washing following hybridization and thereby minimizes both leakage and signal degradation resulting in:
  • Consistent results
  • Fewer steps
  • Ease of use

• Routine use of external controls allow for consistency in assay performance

• Analysis of healthy versus disease cell populations allows for determination of clinical significance
Detection of E6/E7 in cells at different phases of the cell cycle

Cell Cycle stain: Nuclear-ID® Red (Enzo Life Sciences)
Innovative, Cost-Effective Assay for HPV Diagnosis

HPV is one of top 10 targets for clinical testing labs to bring in-house

**RELEVANT**
Detection of E6/E7 mRNA in fixed cell preparation allows for improved diagnosis of the early stages of cervical cancer

**INNOVATIVE**
Able to be combined with probes against other biomarkers or cell analysis dyes to obtain more information in a single test

**OPTIMIZED**
Homogeneous assay reduces the number of handling steps and allows for reduced background and better reproducibility

**VALUE**
Designed to be cost-effective allowing for high margin testing
Questions?

www.enzoclinicallabs.com
800-522-5052