ABSTRACT
Enzo’s newest flow cytometry platform, FLOWSCRIPT™, acts as a “snapshot cell query” by allowing the analysis of mRNA transcript expression in individual cells in a mixed cell population. By studying whether a gene or a set of genes is turned on or off, it is possible to obtain clinically relevant information at the single cell level. The first product developed for use with this technology is the FLOWSCRIPT™ HPV E6/E7 Assay. Integration and ultimately overexpression of HPV oncoproteins, E6 and E7, promotes the growth of malignant cells through the inhibition of tumor suppressors and has been linked with increased likelihood of cervical cancer progression.

INTRODUCTION
The FLOWSCRIPT™ HPV E6/E7 Assay is a flow cytometry-based assay for the detection of mRNA that prescribes the expression of the oncogenic proteins, E6 and E7, produced during infection by high risk HPV types. The FLOWSCRIPT™ HPV E6/E7 Assay is capable of detecting E6/E7 mRNA transcripts from multiple high risk HPV types (HPV16, 18, 31, 33, 35, 45, 51, 52, 53, 54, 56, 58, 59, 68, 82) which together account for over 95% of cervical cancer. This assay employs a novel in situ hybridization technique utilizing a cocktail of oligonucleotide probes specific to multiple targets within the E6 and E7 genes to ensure the detection of these transcripts from most known variants of high risk HPV. Fixed and permeabilized cells are hybridized with these probes and then analyzed by flow cytometry. During hybridization, the probe anneals to the target sequence, thereby emitting a fluorescent label and a quenching molecule whereby no signal is observed in the absence of target. During hybridization, the probe anneals to the target sequence, thereby emitting a fluorescent label and a quenching molecule whereby no signal is observed in the absence of target.

MATERIALS AND METHODS

CATALOG #
DE-200-0001 FLOWSCRIPT™ HPV E6/E7 Positive Control Cells
DE-200-0002 FLOWSCRIPT™ HPV E6/E7 Negative Control Cells
ENZ-110-100 NUCLEAR-ID™ cell fix cell kit (DEFECTIVE) for flow cytometry

Other Reagents Used:
• Formamide (67% by weight, ethanol substituted), Molecular Biology Grade
• Poly Cyclonucleotide Phosphates

Instrumentation:
Though this assay is currently clinically validated for use on the BD Accuri C6, the FLOWSCRIPT™™ Platform Detects Genotypic and Phenotypic Markers Associated with HPV Infection and Cancer Progression

REFERENCES

ASSAY PRECISION NEAR THE LIMIT OF DETECTION

Cytometry A Negative Sample
Cytometry B Negative Sample
Cytometry A Positive Sample
Cytometry B Positive Sample

CONCLUSIONS
Detection of viral mRNA is indicative of viral activity.
Flow cytometry allows analysis of mixed populations on a cell by cell basis, as opposed to other assays such as qPCR which assess a population as a whole.
FLOWSCRIPT™ flow cytometric analysis of HPV E6/E7 mRNA has many advantages:
• Specific Detection of integrated HPV related to cervical disease
• Small sample volumes requirement for analysis
• High throughput testing of up to 25 samples per run
• Multiplexing capability to obtain more information (i.e. cell cycle) in a single test

Flow cytometry allows analysis of mixed populations on a cell by cell basis, as opposed to other assays such as qPCR which assess a population as a whole. FLOWSCRIPT™™ Platform Detects Genotypic and Phenotypic Markers Associated with HPV Infection and Cancer Progression

CLINICAL APPLICATION
This results obtained from the assay were expressed as the percentage of cells in the samples gate that were detected by E6/E7 channel 1. Sensitivity, specificity, and accuracy were calculated using the following standard deviation (SD) testing these results were 0.25, 0.25, and 0.25. E6/E7 results were expressed as the percentage of cells in the samples gate that were detected by E6/E7 channel 1.

The clinical application of the FLOWSCRIPT™ HPV E6/E7 assay is to evaluate the degree of cervical dysplasia present. Results are compared to the literature which shows that E6/E7 testing on cervical samples is a useful differentiating test but it has a low negative predictive value for ASCUS and CIN1 cytology results when compared to biopsies.

Figure 1: Detection of E6/E7 in cells at different phases of the cell cycle

Diagram 1: The flowchart summarizes a light microscope-based assay for the detection of mRNA that prescribes the expression of the oncogenic proteins, E6 and E7, produced during infection by high risk HPV types. The FLOWSCRIPT™ HPV E6/E7 Assay is capable of detecting E6/E7 mRNA transcripts from multiple high risk HPV types (HPV16, 18, 31, 33, 35, 45, 51, 52, 53, 54, 56, 58, 59, 68, 82) which together account for over 95% of cervical cancer.

FLOWSCRIPT™™ Platform Detects Genotypic and Phenotypic Markers Associated with HPV Infection and Cancer Progression

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