HPV E6/E7 mRNA expression analysis using the NovoCyte® Flow Cytometer

Human Papillomavirus (HPV) is the most common sexually transmitted disease and the chief cause of cervical cancer. HPV infects cervical tissues as well as other mucosal and cutaneous regions of the body. Primarily, HPV infection is asymptomatic and transient but it may persist and cause cervical cellular abnormalities. The primary tool for cervical cancer screening, the Pap smear, determines the presence of dysplasia of the cervical squamous epithelium. Widespread screening has been extremely effective for the treatment and prevention of cervical cancer, however, cytology methods of cervical screening require highly trained cytopathologists and do not diagnose HPV infection. Since the discovery of HPV as the underlying cause of cervical cancer, morphologically abnormal cervical cells have been routinely screened for the presence of HPV DNA by PCR analysis. HPV DNA analysis in cervical cancer screening has increased the detection of high-risk lesions with normal cytology results and decreased unnecessary biopsies in women with atypical squamous cells of undetermined significance (ASC-US).

Fluorescent in situ hybridization (FISH) is a powerful technique used for the detection of RNA or DNA in intact cells with a fluorescent probe. Recently FISH technology has been combined with flow cytometry to allow for rapid examination of RNA expression in individual cells. Detection of viral HPV mRNA instead of DNA is a more direct indication of viral activity; therefore the detection of HPV RNA is clinically useful for HPV detection. Overexpression of two HPV oncoproteins E6 and E7 contribute to the malignancy of HPV infected cells by inhibition and degradation of tumor suppressor proteins and promoting cell growth. With new innovations in FISH and flow cytometry, it is possible to detect the presence of E6/E7 HPV mRNA with the use of the FLOWSCRIPT® HPV E6/E7 assay by Enzo Life Sciences and the NovoCyte® flow cytometer providing clinically relevant information at the single cell level. To demonstrate the ability of HPV mRNA detection on the NovoCyte Flow cytometer, both HPV control cell lines and clinical samples were assayed for E6/E7 mRNA transcription and analyzed on the NovoCyte flow cytometer.

HPV RNA detection in cultured cells with the NovoCyte Flow Cytometer

The FLOWSCRIPT HPV E6/E7 assay utilizes in situ hybridization technique with oligonucleotide probes specific for E6 and E7 transcripts. Each probe has a fluorescent label and quencher molecule which ensures that the fluorescent signal is only observed after hybridization of the probe to the target sequence. This fluorescent signal can be easily measured with a flow cytometer. The ability of the NovoCyte flow cytometer to measure HPV RNA transcripts was assessed first in cultured cells. E6/E7 transcripts were measured in positive control cells, which overexpress E6/E7, and negative control cells (Figure 1A). Positive control cells comprise more than 75% of the positive E6/E7 analysis gate while negative control cells make up less than 2%. Next, cultured cells that are known to be HPV negative (Jurkat) and positive (HeLa) were measured for the presence of E6/E7 transcripts (Figure 1B). Jurkat cells (HPV-) comprise <1% of the positive analysis gate while Hela (HPV+) are >95% positive. This data demonstrates the accuracy of measuring HPV transcripts using the NovoCyte flow cytometer in cultured cell lines.

Figure 1. E6/E7 mRNA expression detection on known controls and cell lines. FLOWSCRIPT HPV positive and negative controls were used in the FLOWSCRIPT HPV E6/E7 Assay to detect presence of E6/E7 mRNA expression. Positive control cells overexpression E6/E7 mRNA while Negative Control cells do not express E6/E7. Presence of E6/E7 in a HPV-negative cell line (Jurkat) and HPV-positive cell line (HeLa)
HPV RNA detection in cytology samples with the NovoCyte Flow Cytometer

HPV detection is routinely performed on clinical cytology samples to confirm HPV infection. Therefore, clinical ectocervical specimens were assessed for E6/E7 RNA transcript to ensure accurate detection of HPV E6/E7 mRNA (Figure 2). The clinical threshold is set at 2% of cells within the E6/E7 positive analysis gate determined by previous studies done at Enzo Life Sciences. All HPV positive samples had more than 2% of cells in the analysis gate while all negative samples made up less than 2% of the E6/E7 gate.

Figure 2. E6/E7 mRNA expression detection in known negative and positive clinical ectocervical cells. Clinical cytology samples were evaluated for the presence of E6/E7 mRNA. Representative positive and negative samples are shown. Clinical threshold is 2% cells within the E6/E7 gate. All HPV negative samples were <2% within the E6/E7 analysis gate and all HPV positive samples were >2% of the E6/E7 gate.

Conclusion

HPV detection has been a recent addition to cytology screening for cervical cancer and detection of viral mRNA is a direct indication of viral activity. With the use of the FLOWSCRIPT HPV E6/E7 assay and the NovoCyte flow cytometer, analysis of HPV RNA transcripts can be assessed on a single cell level even in mixed cell populations. Measuring transcripts for HPV RNA is easy on the NovoCyte flow cytometer. Hands-on time is minimized by the use of an optional autosampler (NovoSampler® Pro) which can automatically analyze samples in tubes or plates. Due to the wide dynamic range of detection, there is no need for PMT voltage adjustments limiting the variability between users. With the NovoCyte Flow Cytometer, HPV RNA transcripts detection results are fast and easy to obtain. For additional information please visit:

FLOWSCRIPT® HPV E6/E7 assay
www.enzolifesciences.com
NovoCyte® Flow Cytometer
www.aceabio.com

References


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