

**INTERPRETATION GUIDELINES**

**HPV-16 Status by Real-Time PCR**

**METHODOLOGY/RESULT INTERPRETATION:**

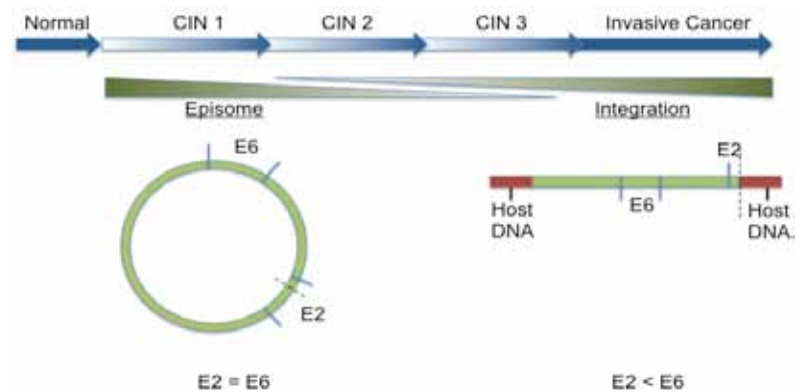
Quantitative, Real-Time PCR is used to measure the number of copies of HPV-16 E2, HPV-16 E6, and human GAPDH genes within a cervical specimen. The quantities of the two HPV-16 genes are compared in the E2/E6 Ratio, which is an estimate of the Viral Status (See Table 1). The quantities of the E6 and GAPDH genes are compared in a copy number ratio (CNR), which is reported as Viral Load (viral genome copies/human genome copies).

**Table 1.** How the E2/E6 Ratio Relates to HPV-16 Integration Status

E2/E6 Ratio	Viral Status
≥ 0.8	Episomal
≥ 0.2 and < 0.8	Mixed
< 0.2	Integrated

**CLINICAL SIGNIFICANCE:**

**Viral Status** is an estimate of the HPV-16 virus integration state. As illustrated in Figure 1, the HPV-16 virus contains a circular DNA genome. When HPV-16 infects a host cell, the viral DNA genome remains circular and distinct from the host genome, a form called an “episome”. In this episomal form, the HPV-16 virus has complete copies of two viral genes, E6 and E2. As HPV-16 infection persists, the viral episome may become integrated into the host DNA genome. In the process of integration, the viral genome is disrupted and the E2 gene may be completely or partially deleted. This integrated form of HPV-16 can be found in up to 80% of HPV-16 positive cervical cancers [1-3].



**Figure 1.** The HPV-16 Viral Genome Status in Cervical Cancer.

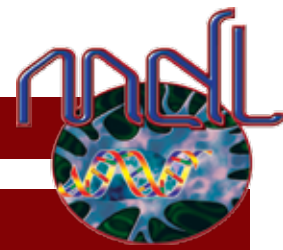
Multiple clinical studies have shown that the episomal form was mostly found in non-progression of preinvasive lesions, whereas the integrated form was found mostly in progression of preinvasive lesions. Thus, a decrease in the episomal form was associated with poorer outcome [4-6].

**Viral Load** is a measurement of the copies of the HPV-16 viral genome per copy of the host genome. Multiple clinical studies have shown that increasing HPV-16 viral load is associated with a persistent infection and progression of preinvasive lesions [4, 6-10]. In addition, it has been shown that, with repeated measurement, decreasing HPV-16 viral DNA load is associated with viral clearance [11]. However, HPV-16 viral load may decrease when the viral DNA has integrated and cannot propagate.

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This test was developed and its performance characteristics determined by this laboratory. It has not been cleared or approved by the Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary. This test is used for clinical purposes. It should not be regarded as investigational or for research. The laboratory is regulated by the Clinical Laboratory Improvement Act of 1988.



## INTERPRETATION GUIDELINES

### HPV-16 Status by Real-Time PCR *continued*

#### METHODOLOGY/RESULT INTERPRETATION:

**E6 variant:** PCR is used to amplify regions of the HPV-16 E6 gene from a cervical specimen. A subsequent multiplex allele specific primer extension (ASPE) reaction detects specific changes at 12 positions of the HPV-16 E6 DNA sequence. The results are compared to the “European prototype”, the first HPV-16 sequence identified from a German woman, to determine the HPV-16 variant. The HPV-16 variants are grouped roughly by geographical relationship and are reported to have an association with the severity of disease, as depicted in Table 1. A result of “N/D” indicates that the HPV-16 variant could not be determined.

#### CLINICAL SIGNIFICANCE:

While there are conflicting reports of the correlation of the European variants and the severity of disease, a study with 354 women in a US population found the age and race/ethnicity adjusted odds ratio was 1.55 (0.68 – 3.55, 95% CI) for risk of carcinoma or its immediate precursor, HSIL-S (high grade squamous intraepithelial lesion, severe dysplasia/CIN3) compared to the European prototype. Of the European variants, the T350G is the most commonly found among invasive cancers, and has been linked to an increased risk for cervical disease progression.

More consistent evidence is found for the increased risk of carcinoma or severe dysplasia associated with the non-European variants. In the same study of the US population the adjusted odds ratio was 3.76 (1.32 – 10.66, 95% CI) for non-European variants compared to the European prototype. A number of other clinical studies have shown that one non-European variant in particular, the Asian-American variant, was associated with CIN3 and invasive cancer with one study reporting up to 20-fold higher prevalence than the European prototype in cervical cancer. Although all of the HPV-16 variants are found in patients with high grade lesions and cervical cancer, the aggregate evidence reflects that the non-European variants, particularly the Asian-American variant, have an increased risk for persistence and progression to cancer.

**Table 1:** HPV-16 Variant Result Description and Interpretation.

Result	Variant Group	Distribution (est., US pop.)	Increased Risk
EP	European Prototype	45 - 50%	
EV(T350G), As	European Variant	35 - 40%	
AF-1/2, NA-1	Non-European Variant	2 - 10%	
AA	Non-European Variant	2 - 10%	

**Abbreviations:** EP, European prototype; EV(T350G), European variant (with T350G mutation); As, Asian variant; AF-1/2, African variants; NA-1, North American variant; AA, Asian-American variant.

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