HPV-16 Risk Assessment Status

High-risk human papillomaviruses (HPV) are found in almost all cervical tumors and are therefore considered a causative factor of cervical cancer. However, most cervical intraepithelial lesions caused by high-risk HPV are known to spontaneously regress. This suggests that, although a causative factor, high-risk HPV infection does not independently cause cervical cancer. Therefore, it is important to consider additional factors or surrogate markers that play a role in the progression of premalignant cervical lesions. Many studies have identified HPV-related markers that can predict the progression of cervical intraepithelial lesions and cervical cancer. The markers include, but are not limited to, HPV type, HPV integration status, HPV viral load, and the HPV-16 E6 genetic variant.

HPV-16: INTEGRATION, VIRAL LOAD, AND CERVICAL CARCINOGENESIS

- Of the high-risk HPV types, HPV-16 is the prevalent genotype associated with approximately 20% of low-grade cervical lesions, 50% of high-grade lesions and 60% of cervical squamous cell carcinomas. HPV-18 is prevalent in approximately 10% of cervical squamous cell carcinomas. Other HPV types (i.e., HPV-26, -31, -33, -35, -39, -45, -51, -52, -53, -56, -58, -59, -66, -68, -73, and -82) have less than a 10% prevalence in cervical squamous cell carcinomas.

- During its normal life cycle, the HPV-16 genome exists in a circular, or “episomal” state and upon infection of the basal cells of the squamous epithelium, remains distinct from the host genomic DNA.

- The HPV-16 genome contains the viral oncogenes E6 and E7, which encode proteins capable of immortalizing and transforming normal cells. The E6 and E7 proteins interfere with host tumor suppressor genes p53 and pRB, respectively. In the episomal state, the expression of E6 and E7 are tightly regulated by a protein encoded by the viral gene E2.

- Cervical neoplastic progression requires the quantitative and spatial deregulation of E6 and E7 expression, i.e., elevated E6 and E7 expression throughout the epithelium as well as the basal epithelial cells.

- In approximately 80% of HPV-16 positive cervical carcinomas, a truncated viral genome which has integrated into the host genomic DNA is found. The integration is correlated to elevated E6 and E7 expression and confers a proliferative advantage to these cells, accounting for their clonal outgrowth.

- In cervical tumors where HPV-16 has integrated into the host DNA, consistently the E2 gene is completely or partially deleted. The loss of E2, which regulates E6 and E7, allows for increased expression of the viral oncoproteins. In addition, the integration leads to stability of the viral E6 and E7 mRNA transcripts, therefore increasing production of the two viral oncoproteins.

- Multiple clinical studies have shown that the episomal form of HPV-16 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.

- In general, clinical studies in several countries show that the integration of HPV-16 is accompanied by an increase in the grade of cervical lesions and is strongly associated with persistent HPV infection and progression of cervical lesions.

- Only the episomal form of HPV-16 is capable of replicating and propagating the viral infection. Episomal replication may lead to a high viral load.

- Integrated HPV-16 is incapable of replicating. Host cells with integrated virus begin to expand and progress toward the cancerous state, while the episomal form is “lost”.

- HPV-16 infections are cleared more slowly than other HPV types and HPV-16 viral load has been reported as a marker for persistent HPV infection. HPV-16 viral DNA load is also reported to be associated with high-grade cervical lesions and invasive cancer.
• Recent clinical studies demonstrate that serial measurement of HPV-16 viral load may be a useful predictor for viral clearance and determining the outcome of viral infection.

• Sequencing of the HPV genome from clinical samples has identified many intratype variants. Those in the HPV-16 E6 gene have been particularly well characterized and are used to identify HPV-16 variants.

• The first HPV-16 sequence was identified from a European woman and was termed the “European prototype” (EP). This sequence is used for comparison of all other HPV-16 sequences from clinical samples.

• Worldwide studies have identified several other major HPV-16 variants that are roughly grouped by geographic relationship.

• The European variants (EV) usually have one or two nucleotide differences from the EP. This group also includes an emerging variant, the Asian variant (As).

• One of the characteristic genetic changes of the EV commonly found in cervical cancer is T350G. The T350G change translates into an amino acid change in the HPV-16 E6 protein. This altered E6 protein has been shown to enhance the transformation of cervical cells more effectively than the E6 protein from HPV-16 EP.

• A study of 354 US women found the HPV-16 EV to have a 1.6 odds ratio for risk of cervical cancer or CIN3 lesions compared to the EP.

• The major non-European variants are the African variants (AF-1 and AF-2), the North American variant (NA-1), and the Asian-American variant (AA).

• A study of 354 US women found the HPV-16 non-European variants collectively to have a 3.8 odds ratio for risk of cervical cancer or CIN3 lesions compared to the EP.

• Several worldwide studies have identified that the AA variant has a particularly high association with cervical cancer and CIN3 lesions with one study finding a 20-fold great association with invasive disease.

Table 1. HPV-16 Variants: Grouping, Distribution, and Risk

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

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.

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


HPV-16 E6 Genetic Variation and Cervical Carcinogenesis

• To understand the fact that only a minority of women infected with HPV-16 develop cervical cancer, there has been interest in the role of HPV DNA sequence variation, and therefore HPV protein structure and function variation, in the progression of HPV-related cervical lesions.
HPV-16 Status Test For Integration, Viral Load, and E6 Variant

- The sample is collected from the endocervix and ectocervix using the OneSwab® specimen collection platform.
- The HPV-16 Status Test can also be performed on samples collected and placed in the ThinPrep® solution.
- MDL offers the HPV-16 Status Test as a reflex for HPV Type-Detect® samples that are HPV-16 positive ONLY.
- The HPV-16 Status Test combines information from two assays, one for integration and viral load and one for genotyping the E6 variant.
- The HPV-16 integration and viral load assay uses quantitative Real-Time PCR to measure the number of copies of HPV-16 E2, HPV-16 E6, and human GAPDH genes within the cervical sample.
- The quantities of the two HPV-16 genes are compared in the E2/E6 Ratio, which is an estimate of the Viral Integration Status (See Table 2).
- 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).

Table 2. How the E2/E6 Ratio Relates to HPV-16 Integration Status

<table>
<thead>
<tr>
<th>E2/E6 Ratio</th>
<th>Viral Status</th>
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<tbody>
<tr>
<td>≥ 0.8</td>
<td>Episomal</td>
</tr>
<tr>
<td>≥ 0.2 and &lt; 0.8</td>
<td>Mixed</td>
</tr>
<tr>
<td>&lt; 0.2</td>
<td>Integrated</td>
</tr>
</tbody>
</table>

- The HPV-16 variant assay uses PCR and multiplex allele specific primer extension (ASPE) to identify specific changes at 12 positions of the HPV-16 E6 gene and determine the variant.

Advantages of the HPV-16 Status Test

- Unique molecular testing that supplements the information provided by HPV Type-Detect® providing an integrated view of four factors reported to be associated with the development of HPV-16-related cervical dysplasia and cervical cancer.
- The Real-Time PCR method is sensitive and specific and is capable of accurately quantifying the copies of HPV-16 E2, HPV-16 E6, and human GAPDH genes present in OneSwab® and ThinPrep® samples.
- The PCR and ASPE method is sensitive and can specifically identify known nucleotide changes at multiple positions in the HPV-16 E6 gene, rapidly identifying HPV-16 E6 variants.
- Provided and supported by Oncoveda Cancer Research Center™, established by Medical Diagnostic Laboratories, L.L.C. to translate cancer research into diagnostic tools.

References


