



**CPAL**

Central Pennsylvania Alliance  
Laboratory

# Technical Bulletin

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## New Test - HPV DNA Test

**Starting Date:** March 10, 2003

**Medical Use:** The human papillomavirus (HPV) has been clearly established as the primary cause of cervical cancer in nearly all cases.<sup>1</sup> Testing for HPV can help with triaging women with atypical squamous cells of undetermined significance (ASCUS or ASC). The sensitivity of HPV DNA testing for the detection of biopsy-confirmed high grade squamous intraepithelial lesion (HSIL) in women with ASC is 83% to 100% and is higher than the sensitivity of a single repeat cervical cytological test in all of the reported series. The negative predictive value of DNA testing for high-risk types of HPV is generally reported to be 98% or greater.<sup>2</sup> The 2001 Consensus Guidelines for the Management of Women With Cervical Cytological Abnormalities states: "Women with ASCUS should be managed using a program of 2 repeat cytology tests, immediate colposcopy, or DNA testing for high-risk types of human papillomavirus. Testing for HPV DNA is the preferred approach when liquid-based cytology is used for screening".<sup>2</sup>

There are 23 distinct HPV types which are specific to the genital tract of both males and females. Clinical manifestation is dependent upon epithelial location, HPV type and host immune status. Viral DNA of HPV type 16 and type 18 have been found in 60% and 20% of cervical carcinomas, respectively.<sup>3</sup> Historically, they are regarded as high-risk cancer associated HPVs. HPV types 31, 33, and 35 are more frequently detected in HSIL. These have demonstrated an intermediate cancer association. Additional HPV DNA types 45, 51, 52, 56, 58, 59, and 68 have been identified as the principal HPV's detectable in the remaining HSIL lesions and in low grade SIL.<sup>4</sup> Currently, high-risk HPV types refer to HPV 16/18/31/33/35/39/45/51/52/56/58/59/68.<sup>5</sup>

**Method:** Digene HC2 High-Risk HPV DNA Test

The HC2 High Risk HPV DNA Test is a nucleic acid hybridization assay with signal amplification. Specimens containing the target DNA hybridize with a specific HPV RNA probe cocktail. The resultant RNA:DNA hybrids are captured onto the surface of a microplate well coated with antibodies specific for RNA:DNA hybrids. Immobilized hybrids are then reacted with antibodies conjugated with multiple alkaline phosphatase molecules to achieve a substantial signal amplification in the subsequent step of light production. The intensity of the light emitted denotes the presence or absence of target DNA in the specimen. A relative light units (RLU) measurement equal to or greater than the Cutoff Value indicates the presence of HPV DNA sequences in the

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specimen. An RLU measurement less than the Cutoff Value indicates the absence of the specific HPV DNA sequences tested or HPV DNA levels below the detection limit of the assay.

Digene's High-Risk HPV Probe contains a cocktail of RNA probes for HPV 16/18/31/33/35/39/45/51/52/56/58/59/68.

### **Specimen Collection:**

Specimens collected using the Digene Cervical Sampler (Digene Cervical Brush and Specimen Transport Medium) or specimens collected using a broom-type collection device and placed in Cytoc PreservCyt Solution are the only acceptable specimen types. Specimens should be collected in the routine manner and the ThinPrep Test Slides should be prepared according to Cytoc instructions. Cervical specimens must be collected prior to the application of acetic acid or iodine if colposcopic examination is being performed.

#### Specimens In Cytoc PreservCyt Solution:

There must be at least 4 mL of PreservCyt Solution remaining after the ThinPrep slide is prepared for the HC2 HPV DNA Test. Samples with less than 4 mL after the ThinPrep slide has been prepared may contain insufficient material and could be falsely negative in the HC2 HPV DNA Test. PreservCyt Solution specimens may be held for up to 3 weeks following collection at temperatures between 4<sup>0</sup> C and 37<sup>0</sup> C prior to processing for the HC2 HPV DNA Test. PreservCyt Solution specimens cannot be frozen.

#### Specimens In Digene Specimen Transport Media:

Specimens collected using the Digene Cervical Sampler may be held for up to 2 weeks at room temperature, after which specimens can be stored an additional week at 2-8<sup>0</sup> C. If the assay will be performed more than 3 weeks from collection, specimens can be frozen at -20<sup>0</sup> C for up to 3 months prior to testing.

**Cautions:** The HC2 High Risk HPV DNA Test can detect high/intermediate-risk HPV types 16/18/31/33/35/39/45/51/52/56/58/59/68, but cannot determine the specific HPV type present.

The use of this test is indicated:

1. To aid in the diagnosis of sexually transmitted HPV infections with HPV types 6, 11, 16, 18, 31, 33, 35, 39, 42, 43, 44, 45, 51, 52, 56, 58, 59, and 68.
2. To screen patients with ASCUS (atypical squamous cells of undetermined significance) PAP smear results to determine the need for referral to colposcopy. The results of this test are not intended to deter women from proceeding to colposcopy.

2 of 3

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York, Pa. 17403

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3. In women with LSIL or HSIL Pap smear results, prior to colposcopy, an HC2 HPV result will aid the physician in patient management by assisting with risk assessment for SIL. This result is not intended to deter the patient proceeding to colposcopy.

**Note:** CPAL will offer only the high risk HPV DNA test. If both low risk and high risk HPV DNA testing is requested, the entire specimen will be forwarded to Nichols-Chantilly (AML/Quest).

- References:**
1. Walboomers JMM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol.* 1999; 189:12-19.
  2. Wright TC, Cox JT, Massad LS, Twiggs LB, and Wilkinson EJ, 2001 Consensus Guidelines for the Management of Women With Cervical Cytological Abnormalities, *JAMA*, 2002(16); 287: 2120-2129.
  3. Pfister H, The role of human papilloma virus in anogenital cancer. In Lorincz AT, Reid R, editor. *Human papillomavirus 1*. 2<sup>nd</sup> ed. *Obstet Gynecol Clin NA* 1996;23:579-595.
  4. Lorincz AT, Reid R, Jenson, AB, Greenberg MD, Lancaster W, Kurman RJ, Human papillomavirus infection of the cervix: relative risk associations of 15 common anogenital types. *Obstet Gynecol* 79:328-337; 1992.
  5. Digene Hybrid Capture 2 High Risk HPV DNA Test package insert, 2/2002.

**Laboratory Contacts:**

Lu Song, Ph.D. , Technical Director, CPAL Lab: 717-851-1422

Peter C. Côté, M.D., Medical Director, CPAL Lab: 717-738-6114