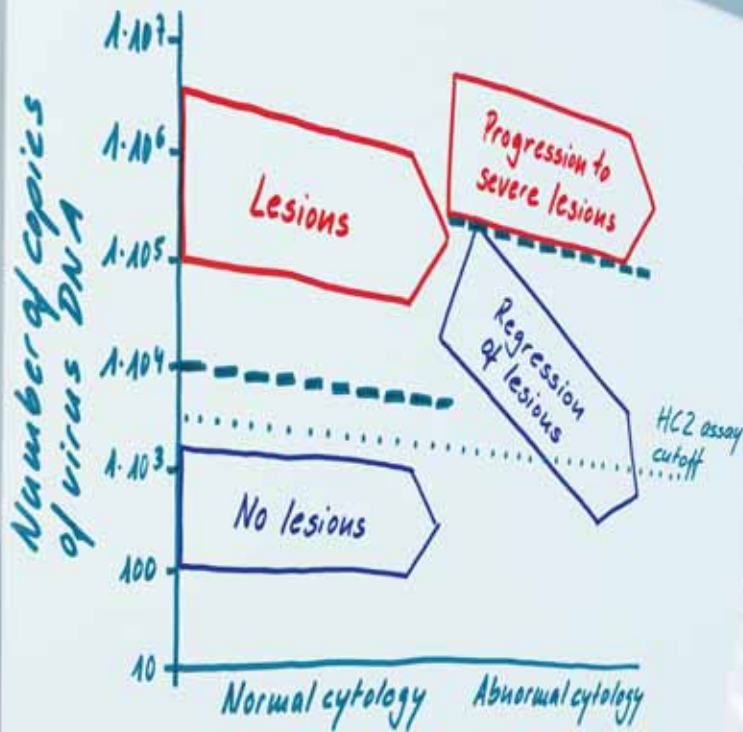


HPV DNA Testing: Clinical vs. Analytical Sensitivity

The *digene* HPV Test



How to validate an HPV test for optimal clinical performance

Clinical sensitivity more accurately represents an assay's ability to diagnose cervical disease

While analytical sensitivity is decisive for HIV or hepatitis C tests, it is much less significant in evaluating a test for the detection of HPV DNA. Testing platforms with high analytical sensitivity (e.g., PCR-based tests) may yield positive results which are clinically irrelevant and, thus, misleading.

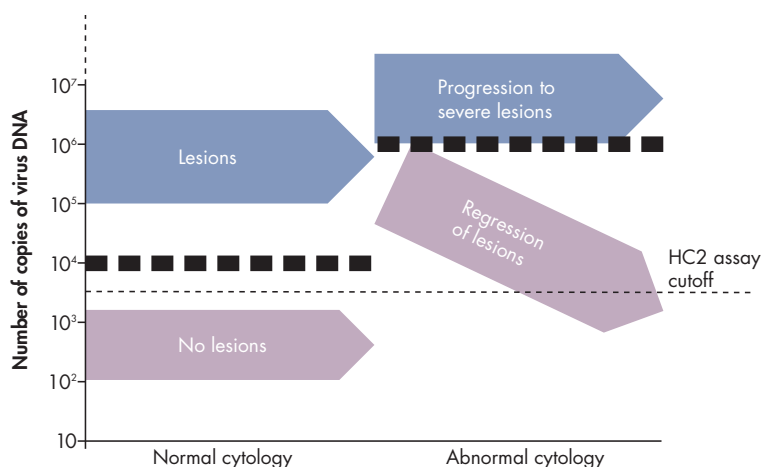


Figure 1. Clinical relevance of a cutoff value in relation to progression risk for CIN+.

Adapted from Snijders P.J.F. et al. (1). The thick dashed lines indicate the informative viral load thresholds.

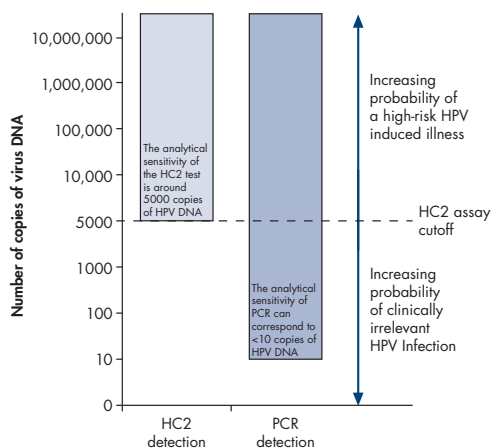


Figure 2. Significance of the HC2 assay cutoff. This figure compares the clinical relevance of the *digene* HPV HC2 DNA Test and PCR-based methods. The **HC2 assay cutoff** value differentiates clinically irrelevant HPV infections from those that are far more likely to lead to high-risk HPV-induced disease (1).

For HPV testing, clinical sensitivity is considerably more important: the goal is to identify as positive only high-risk HPV infections that actually present a likelihood of developing cervical cancer. The test procedure must define a validated threshold value differentiating clinically relevant HPV infections from an HPV DNA presence that does not correlate to future cervical disease (Figure 1).

The *digene*® HPV HC2 DNA Test (based on Hybrid Capture® 2 technology) is the **only** HPV test procedure enabling physicians to estimate the clinical relevance of high-risk HPV infection using a validated threshold (cutoff) value specifying the number of DNA copies above which a test is regarded as positive. The *digene* HPV HC2 DNA Test's cutoff is approximately 5000 copies of HPV DNA. To date, all PCR methods lack this threshold value, which is critical for patient management (Figure 2).

The *digene* HPV HC2 DNA Test is the most extensively validated standardized HPV test

Studies involving over 300,000 patients (2, 3, 4) have been conducted worldwide using the *digene* HPV HC2 DNA Test. Additionally, numerous studies have employed a range of designs and clinical end-points to determine sensitivity in detecting clinically relevant infections with high-risk HPV — including dysplasia, pre-cancerous stages, and cervical cancer. Twenty-six selected studies demonstrate that results obtained with the *digene* HPV HC2 DNA Test are superior in clinical sensitivity when compared to PCR methods (Figure 3).

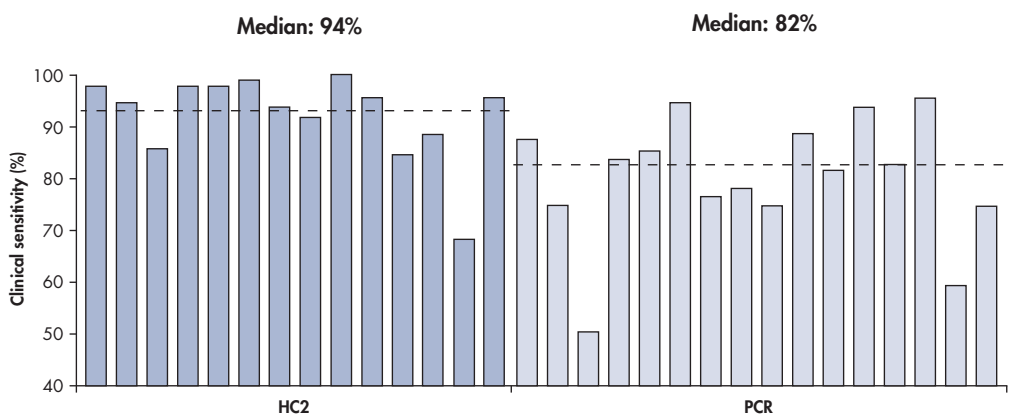


Figure 3. Results, per study, of HC2 vs. PCR clinical sensitivity. This figure shows the results of each study’s measurement of the *digene* HPV HC2 DNA Test and PCR methods in recognizing pre-cancerous stages (CIN 2+). See reference 5.

These studies, in total, found that the *digene* HPV HC2 DNA Test yielded results with a median of 94% clinical sensitivity. In contrast, PCR procedures showed a clinical sensitivity of only 82%. The *digene* HPV HC2 DNA Test consistently provided superior sensitivity in detecting pre-cancerous stages — required for both primary screening and triage risk assessment (Figure 4).

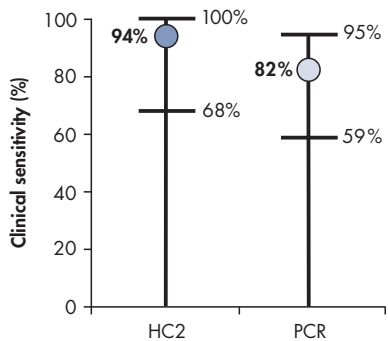
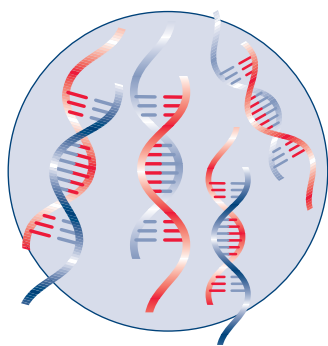
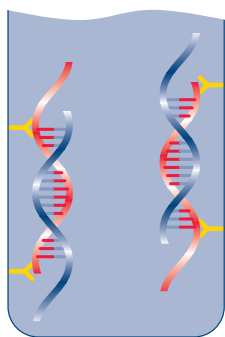


Figure 4. Summary of HC2 vs. PCR clinical sensitivity. Numbers not bold-faced indicate highest and lowest sensitivities found in the studies (5).

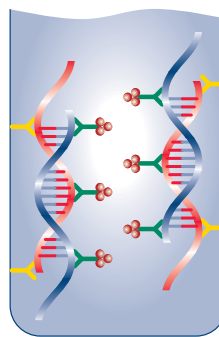
The ***digene* HPV HC2 DNA Test** detects the presence of 13 high-risk HPV types — using full-length RNA probes complementary to the HPV DNA, special antibodies, detection reagents, and chemiluminescence. Sample collection is similar to a Pap test, taking a smear of cells from the cervix. The cells in the sample are denatured and the HPV DNA is split into single strands. The basic assay steps are outlined below.



Hybridize RNA probe with target DNA. Target DNA combines with specific RNA probes, creating RNA:DNA hybrids.



Hybrid capture. RNA:DNA hybrids are captured onto a solid phase coated with universal capture antibodies specific for RNA:DNA hybrids.



Signal amplification. Captured RNA:DNA hybrids are detected with multiple antibodies conjugated to alkaline phosphatase. The signal resulting from the chemiluminescent reaction is read and results interpreted.

Signal strength is proportional to the HPV load in the sample. The clinically validated cutoff value of the *digene* HPV HC2 DNA Test is 1 pg/ml or approximately 5000 viral copies/sample. Samples with lower numbers of virus copies are classified as negative. The *digene* HPV HC2 DNA Test provides the highest clinical sensitivity required for the diagnosis of HPV and patient management.

In contrast, in PCR, primers bind to and amplify defined areas of virus DNA presenting a number of disadvantages (Table 1). Due to the absence of a threshold value for PCR-based tests, the number of clinically irrelevant positive results may increase. Additionally, and critically, PCR can signal false negatives in the presence of advanced disease (6). Most PCR methods are based on primers targeting L1 or E1 regions of the HPV genome. These targeted segments may be eliminated when virus DNA is integrated into the human genome — a precursor for many advanced stages of the disease. Many PCR methods yield false-negative results that do not recognize a proportion of disease stages such as severe dysplasia and cervical carcinoma.

The *digene* HPV Test

The *digene* HPV HC2 DNA Test is a primary tool for detecting only clinically relevant HPV infections

The determination of whether a high-risk HPV infection may lead to cervical cancer is highly dependent on the number of detected copies of virus DNA. Only the *digene* HPV HC2 DNA Test provides these clinically significant results, and remains effective throughout all stages of disease progress.

Table 1. HC2 and PCR: point-by-point comparison

	HC2	PCR
CE-marked and FDA-approved	Yes	No
Clinical performance	94% clinical sensitivity	82% clinical sensitivity
Standardized method	Yes	No
Risk of false negatives due to inhibition	No	Yes
Risk of false negatives due to L1 or E1 gene deletions	No	Yes
Throughput	Up to 264 tests in 8 hours	Depends on method
Data used to support cervical cancer guidelines	Yes	No
High-risk type profile	Currently accepted clinically relevant types 16,18,31,33,35,39,45, 51,52,56,58,59,68 (7)	Depends on method

Ordering Information

Product	Contents	Cat. no.
<i>digene</i> HPV HC2 DNA Test	96 tests for 40 cervical samples*	5196-1330
<i>digene</i> High-Risk HPV HC2 DNA Test	96 tests for 88 cervical samples†	5197-1330

* Includes probe diluent, high-risk probes, quality controls, calibrator, capture microplate, reagents, and buffers.

† Includes probe diluent, high-risk and low-risk probes, quality controls, calibrator, capture microplate, reagents, and buffers.

The *digene* HPV HC2 DNA Test and *digene* High-Risk HPV HC2 DNA Test are intended for in-vitro diagnostic use.

Visit www.qiagen.com/hpv for further information.

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References

1. Snijders, P.J.F., van den Brule, A.J.C., and Meijer, C.J.L.M. (2003) The clinical relevance of human papillomavirus testing: relationship between analytical and clinical sensitivity. *J. Pathol.* **201**, 1.
2. Lorincz, A.T. (2006) HPV testing by hybrid capture. In: Monsonago, J., ed. *Emerging Issues on HPV Infections: From Science to Practice*. Basel: Karger, p 54.
3. Fetterman, B., Shaber, R., Pawlick, G., and Kinney, W. (2005) Lessons from practice: the first hundred thousand Pap and HPV cotests for general population screening. Poster presented at the 22nd International Papillomavirus Conference and Clinical Workshop. Vancouver.
4. Ronco, G. et al. (2008) Results at recruitment from a randomized controlled trial comparing human papillomavirus testing alone with conventional cytology as the primary cervical cancer screening test. *J. Natl. Cancer Inst.* **100**, 492.
5. Lorincz, A.T. and Smith, J.S. (2006) Sexually transmissible viral pathogens: human papillomaviruses and herpes simplex viruses. In: Lorincz, A.T., ed. *Nucleic Acid Testing for Human Disease*. Boca Raton: Taylor & Francis Group/CRC Press, p 243.
6. Morris, B.J. (2005) Cervical human papillomavirus screening by PCR: advantages of targeting the E6/E7 region. *Clin. Chem. Lab. Med.* **43**, 1171.
7. Wright, T.C. Jr. and Schiffman, M. (2003) Adding a test for human papillomavirus DNA to cervical-cancer screening. *N. Engl. J. Med.* **348**, 489.

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