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Comparative Evaluation of First- and Second-Generation Digene Hybrid Capture Assays for Detection of Human Papillomaviruses Associated with High or Intermediate Risk for Cervical Cancer

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In the present study we comparatively evaluated the first- and second-generation Digene Hybrid Capture assays for detection of human papillomaviruses (HPV) associated with high or intermediate risk for cervical cancer in cervical specimens. Concordant results were obtained with 468 of 483 (96.8%) specimens. All 15 specimens which gave repeatedly discordant results were positive by the second-generation test only, and 14 of them tested PCR positive. The enhanced sensitivity of the second-generation assay is mainly a result of the reformulation of hybridization reagents and, to a lesser extent, a result of the addition of new HPV probes.

The strong association between infection with certain human papillomavirus (HPV) types and cervical cancer that has emerged in the past 10 years has brought new urgency to the need for establishing accurate methods to diagnose HPV infection. Although cytological screening by the Papanicolaou test, or Pap smear, continues to play a major role in the early diagnosis of cervical neoplastic lesions, assays that detect HPV DNA in cervical smears may represent an important source of adjunct information for the purposes of counselling, selection of therapy, and follow-up (18).

Several HPV DNA detection methods have been described during the last decade, each of which allows the detection of a wide spectrum of HPV types, but none has fulfilled all expectations (reviewed in reference 6). Amplification-based methods, mainly PCR, are currently the most sensitive methods for detection of HPV DNA. They are ideal instruments for research and epidemiological purposes since they allow the detection of low-viral-load infections and also minimize the risk of misclassification of HPV infection status (6). However, due to frequent contamination problems and consequent false-positive results and the present unacceptably high costs of amplification technology, they are not currently readily applicable to diagnostic laboratories for the routine detection of HPV infection (6, 18).

In order to overcome these problems, the first-generation Hybrid Capture System DNA detection test (Digene Diagnostic, Silver Spring, Md.) for the rapid detection of genital HPV types in clinical settings was developed a few years ago (13). In this assay, exfoliated cells are treated with alkali-denaturate solution, and the processed samples are hybridized under high-stringency conditions in a solution containing two mixtures of single-stranded RNA probes: one for HPV types 6, 11, 42, 43, and 44, associated with a low risk for cervical cancer, and one for HPV types 16, 18, 31, 33, 35, 45, 51, 52, and 56, associated with a high or intermediate risk for cervical cancer (referred to in this work as low-risk and indeterminate- or high-risk HPV types, respectively). Positive specimens are detected by binding the hybridization complexes to tubes coated with a monoclonal antibody to RNA-DNA hybrids. The bound hybrids are detected by the addition of an alkaline phosphatase-conjugated antibody to RNA-DNA hybrids, followed by the addition of a chemiluminiscent substrate. The emission of light is measured as relative light units (RLU) in a luminometer (13). The analytical sensitivity of the assay was estimated as 50,000 copies of HPV 16 DNA (4, 13). Several studies have evaluated the clinical sensitivity and specificity of the first-generation assay mainly against PCR and histology (2–5, 7, 9, 10, 14–17). However, as expected, the first-generation Hybrid Capture assay was less sensitive than PCR and other amplification techniques, but its specificity and positive predictive value were higher than those of PCR. To improve the sensitivity of the Hybrid Capture assay, the Digene Corporation has recently modified the first-generation assay that has been approved by the U.S. Food and Drug Administration. The improved second-generation assay (Digene HPV Test Hybrid Capture II) was launched in Europe at the beginning of 1998. According to the manufacturer's statement, in the second-generation assay analytical sensitivity was increased to 1,000 HPV DNA copies by the reformulation of hybridization reagents and by the addition of new probes for four high- or intermediate-risk HPV types, 39, 58, 59, and 68. Additionally, microtiter plates replaced the hybridization tubes used in the first-generation test.

In the present study we comparatively evaluated both generations of Hybrid Capture assay for detection of high- or intermediate-risk HPV in a total of 483 random consecutive cervical specimens obtained from the same number of women with abnormal Papanicolaou screening test results. All specimens were collected with the Digene Specimen Collection Kit and tested by both assays in parallel according to the manufacturer's instructions, as previously described (14). The evaluation was performed in a double-blinded manner. According to the manufacturer's interpretation criteria, specimens with RLU/cutoff value ratios of $\geq$1.0 were considered positive for one or more high- or intermediate-risk HPV types, and samples with RLU/cutoff value ratios of $<$1.0 were considered negative for the HPV types tested.

Concordant HPV DNA results were obtained in 468 of 483

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(96.8%) cervical specimens. Thus, 193 cervical specimens tested negative and 275 specimens tested positive for high- or intermediate-risk HPV types by both assay versions. In total, 15 cervical specimens gave repeatedly discordant results, and all were positive by second-generation assay only. For 12 specimens with discordant results the RLU/cutoff value ratios obtained in the second-generation assay ranged from 2.8 to 8.9. For the remaining three specimens with discordant results the RLU/cutoff value ratios obtained were 28.32, 97.23, and 439.29, respectively. To resolve the HPV DNA status for the discordant specimens, all 15 specimens were additionally tested in duplicate by an HPV L1 consensus primer MY09/MY11 PCR-based test, which was originally developed by Manos et al. (8) and modified as previously reported by our group (11). Altogether, 14 of 15 discordant specimens tested clearly HPV DNA PCR positive. A 536-bp fragment of the beta-globin gene was successfully amplified from all 15 discordant specimens. Seven discordant specimens contained HPV type 16, two contained HPV type 56, one contained HPV type 18, one contained HPV type 31, one contained HPV type 35, one contained HPV type 58, and one contained HPV type 68, as determined by restriction fragment analysis of the MY09/MY11 PCR products with seven restriction enzymes (1). Interestingly, in all discordant samples containing HPV DNA types covered by the hybridization probes included in both assay generations (HPV types 16, 18, 31, 35, and 56) the RLU/cutoff value ratios obtained in the second-generation assay were below 28.32 (in 11 of 12 samples they were below 8.9), which would indicate that the low HPV DNA levels present in these samples produced false-negative results in the first-generation assay. By contrast, however, both discordant specimens with relatively high RLU/cutoff values (97.23 and 439.29) contained HPV DNA types covered by hybridization probes included in the second-generation assay only (HPV types 58 and 68), suggesting that the lack of appropriate probes in the first-generation assay hybridization solution produced false-negative results with these specimens. A specimen obtained from a 36-year old woman with a cervical intraepithelial neoplasia, grade 1, lesion—which produced repeatedly positive results by the second-generation assay, giving RLU/cutoff values from 2.3 to 4.1, and which was negative both by the first-generation assay and by MY09/MY11 HPV PCR—was additionally tested with two other HPV consensus primers and four type-specific primer sets, as described previously (12). All six additional PCRs gave negative results, suggesting a false-positive result by the second-generation assay for this sample.

According to the results of our study, the second-generation Digene Hybrid Capture HPV DNA assay is more sensitive than its first-generation version. The enhanced sensitivity of the second-generation version is mainly a result of the reformulation of reagents and, to a lesser extent, a result of the addition of new HPV probes. The second-generation test is more rapid and easier to perform and thus more appropriate for use in the diagnostic microbiology laboratory for routine detection of HPV infection.

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