

# Update on recent clinical studies using HPV testing for screening and diagnosis of cervical neoplasia

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**ABSTRACTS** Because HPV is the primary etiologic agent in cervical cancer, HPV testing may be useful for cervical cancer screening. Among the assays measuring HPV DNA, polymerase chain reaction (PCR)-based testing is the current research reference standard because it is highly sensitive and detects a large number of different HPV types. However, the extremely sensitive PCR-based testing may result in undesirable non-specificity in the context of cervical cancer screening, because many young, sexually active normal women harbor low-level, transient HPV infections. Therefore, other methods have been developed for clinical diagnostic use. In the U.S., the leading HPV diagnostic assay is the Hybrid Capture Tube test (HCT), an FDA-approved signal-amplification test that captures and detects target HPV DNA, bound by RNA probes, using antibodies directed against the DNA-RNA hybrids. To evaluate the performance of HCT as well as the MY09-MY11 PCR-based method, we analyzed HPV-D-

NA test data from our cohort of nearly 23,800 women in Portland, Oregon.

Among the 596 samples tested by both PCR and HCT, agreement on overall HPV positivity (any type) was 93%. Using PCR as the reference standard, the sensitivity of HCT was higher for samples obtained from women diagnosed with concurrent squamous intraepithelial lesions (SIL) (81%) than for those collected from women with normal cytology (47%). Quantitative HCT results revealed a higher load of HPV infection among infected women with SIL (mean = 543.2 pg/ml, 95% CI 365.7-720.7) than those with normal cytology (mean = 243.9 pg/ml, 95% CI 95.9-391.9).

In another larger subset of women tested by either assay, the individual performances of HCT and PCR were again more equivalent among women with concurrent SIL than among cytologically normal women. Overall, a higher proportion of women who tested HPV-positive by HCT were found to have concurrent SIL, compared to that of women who tested positive by PCR. On the other hand, women who tested HPV-negative by PCR were less likely to have concurrent or incipient (develop into SIL in a few months) SIL than those tested negative by HCT. Digene Corporation has recently modified HCT by introducing a new microplate format (HC II) and by lowering the detection threshold from the original 10 pg/ml to 1 pg/ml (or even lower) to increase analytical sensitivity of HPV detection. The new HC II kits should be carefully evaluated, along with recently developed PCR diagnostic kits, to determine the most suitable uses of these modified assays in different clinical settings.

**Key words** HPV, assay, screening, diagnosis, cervical neoplasia

**INTRODUCTION** Over the past 15 years, it has become clear that infection with human papillomavirus (HPV) accounts for the vast majority of cervical squamous intraepithelial lesions (SIL) (1) and invasive carcinomas (2). Detection of HPV, particularly those types that are found in carcinoma ("cancer-associated types"), correlates with concurrent and incipient (develop into SIL in just a few months) SIL (3-4). These findings suggest that conventional cervical cancer screening using cytology might be improved by selective use of HPV testing (5-6)

HPV DNA hybridization assays can be broadly categorized into three groups according to method of testing including: direct or non-amplified (such as Southern blot and dot blot), DNA-target-amplified (PCR-based methods), and post-hybridization signal-amplified (e.g. the Hybrid Capture Tube Test, HCT). The latter two groups of assays are widely used

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for both epidemiologic and clinical studies. Because of its ability to amplify HPV DNA exponentially, PCR is the most sensitive assay for detecting of HPV DNA, but it is prone to non-specificity in cervical cancer screening because many clinically normal women test positive only with this highly sensitive method. On the other hand, HCT was designed to achieve high clinical specificity, but may suffer from limited sensitivity.

This update summarizes some of our recent clinical data on general screening using PCR and HCT. In Portland, Oregon, we have conducted a natural history study of HPV infection and cervical neoplasia in a cohort of 23,800 women in which HPV DNA testing was performed on cervico-vaginal lavage samples collected from women before and/or at the time of an SIL diagnosis. The performances of PCR and HCT methods were evaluated.

#### METHODS

**SUBJECTS** Details of the study design have been presented elsewhere (1). Briefly, women aged 16 to 74 years attending one of seven Kaiser-Permanente clinics in Portland, Oregon between April, 1989 and November, 1990 for routine Papanicolaou (Pap) smear examination were invited to participate in a prospective study of cervical neoplasia. The participation rate was approximately 90%. Participants returning for annual Pap smear screening were passively followed for a median of about 4 years. The cervical lavage samples were collected at enrollment as well as selected follow-up visits.

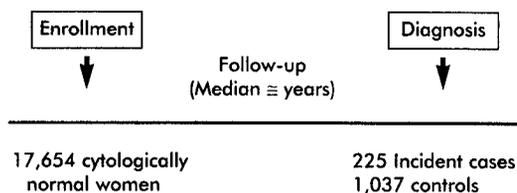
**HPV TESTING** The samples were tested using PCR-based methods and/or HCT. For PCR, the L1 consensus primer pair, MY09 and MY11, along with HMB01 were used to amplify the HPV DNA (7). The amplified products were then hybridized with a generic HPV probe mixture to determine positivity, and with type-specific oligonucleotide probes to identify individual HPV types (7-8). The PCR testing was designed to detect at least 26 HPV types: 6/11, 16, 18, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 66, 68, 73, PAP155, PAP291, and W13B.

Specimens tested by HCT were first treated with sodium hydroxide to hydrolyze specimen RNA and to denature the DNA. The liberated DNA was hybridized in solution with cocktails of full length RNA probes directed against various combinations of HPV types. Subsequently, samples were placed into tubes coated with polyclonal antibodies to the RNA-DNA hybrids, resulting in the solid phase immobilization of hybrids composed of HPV DNA probes in positive samples. The hybrids were detected by an alkaline phosphatase-conjugated monoclonal antibodies to the RNA-DNA hybrids. A dioxetane-based chemiluminescent compound was finally added as a substrate for alkaline phosphatase and pro-

duced light, readable by a luminometer, that corresponded to the amount of HPV DNA. Results of HCT were expressed in picogram/milliliter (pg/ml). Type-specific positivity with HCT corresponded to a cutpoint (or threshold) of approximately 10 pg/ml, although the threshold of positivity varied about two-fold by HPV type. Ten pg/ml of HPV DNA corresponds to approximately 1 million copies per ml, or 100,000 HPV genomes per 100 ul HCT test. HCT was designed to detect 16 HPV types (6/11, 16, 18, 31, 33, 35, 39, 42, 43, 44, 45, 51, 52, 56, and 58).

**ANALYSIS** HPV test results are presented from two overlapping subsets of study subjects. One subset included the subjects whose cervical cell samples were tested by both PCR and HCT (9). Prevalence of HPV positivity was computed for each assay by concurrent cytologic diagnosis. Direct comparisons between the two assays were restricted to the 14 HPV types detectable with both PCR and HCT. We also present data from a larger overlapping subset, a nested incident case-control study. Details of this incident case-control study will be presented in full elsewhere (10). Briefly, as shown in *Figure 1*, cervical cytologic samples from all cytologically normal women in the cohort (n = 17,654) were collected at the enrollment of the study. These women were passively followed for an average of approximately 4 years. During the follow-up, those who developed new SIL were identified as cases. At the diagnosis of an index case, up to 4 controls were selected from those who remained cytologically normal. Whenever possible, another cervical sample was collected from the cases and controls at the time the index case was diagnosed with SIL. Cervical samples of cases and controls collected at enrollment and diagnosis were tested for HPV DNA with PCR and/or HCT. The individual performances of PCR and HCT, separately, are presented at the two times of sample collection. We comment on the likely clinical uses of the two different assays based on the presented data.

Figure 1. Specimen collection in the nested incident case-control study



#### RESULTS

**METHODOLOGIC STUDY (SPECIMENS TESTED WITH BOTH PCR AND HCT)** In the first subset, consisting of the 596 cervical samples that were tested by both PCR and HCT, PCR detected more HPV infections than HCT in the same population. Overall, HPV DNA was detected in 22.5% of the specimens by PCR,

compared to 13.8% with HCT. Restricted to the 14 types detectable by both PCR and HCT, HPV detection with PCR decreased to 18.0%, whereas HCT detected HPV in 13.3% of subjects. When stratified by disease status, both PCR and HCT detected HPV DNA more frequently among the specimens from women diagnosed with concurrent SIL than that from women with normal cytology, with PCR detecting more HPV infection than HCT. Specifically, using PCR results as the reference standard, the sensitivity of HCT in detecting HPV was higher among specimens from SIL cases (81%) than among cytologically normal women (47%) ( $p < 0.05$ ). As described more fully elsewhere (9), the viral loads in HPV-positive samples from women with concurrent SIL diagnoses (mean = 543.2 pg/ml, 95% CI 365.7-720.7) were higher than from those who were cytologically normal (mean = 243.9 pg/ml, 95% CI 95.9-391.9). Since HCT requires higher HPV viral load to identify HPV positivity, the sensitivity of HCT relative to PCR increased among women with cytologic abnormalities in whom viral loads were higher, compared to the sensitivity among cytologically normal women.

Among women tested with both HPV assays, PCR and HCT results agreed in 93%, but 81% of these reflected concordant negative results. Because the majority of samples tested negative with PCR and HCT, the differential performance of the two tests is masked when a simple percentage of agreement is presented. Therefore, we recalculated agreement restricted to the specimens which tested positive with at least one method. In this subset, when restricting to the 14 types detectable by both assays, HPV was detected with both methods in 62.6% of samples. When further stratified by disease status, a 42.9% agreement was observed among the specimens collected from women who were concurrently cytologically normal, while 77.3% agreement was seen among the samples from women with concurrent SIL. Among the 72 samples that tested HPV-positive by both assays, 93% yielded agreement on at least one HPV type. Infection with multiple types of HPV was common. Only 58.3% of the 72 positive samples were found to agree completely on all types detected, despite the restriction to the 14 individual types of HPV detectable by both methods.

**NESTED INCIDENT CASE-CONTROL STUDY** In the nested incident case-control study, women were tested by PCR (earlier period of the study) and/or HCT. In this subset, the performances of the two assays were evaluated individually in samples collected both before and at the diagnosis of SIL. The conclusions regarding assay performance in this subset were consistent with those in the direct comparison presented above. Specifically, stratified analyses by the time of sample collection (at enrollment versus at diagnosis) confirmed that PCR was more sensitive for SIL than HCT, particularly in specimens collected prior to diagnosis of abnormal cytology. Res-

tricted to the 14 HPV types that could be detected by both PCR and HCT, at enrollment, the prevalence of HPV positivity was 40.1% by PCR among those who subsequently developed SIL, compared to 16.7% by HCT. In particular, the prevalence of HPV infection by PCR among those who had subsequent incident HSIL was 43%, while among the 33 incident HSIL cases tested by HCT, only 2 (6%) were found with prior HPV infection. Among the controls at enrollment, approximately 13% and 6% tested HPV-positive by PCR and HCT, respectively.

The HPV positivity of concurrent SIL, based on samples collected at diagnosis of cytologic abnormality, was comparable for PCR (72.6%) and HCT (66.4%), restricted to the 14 types detectable with both assays. By either HPV assay, the prevalence of HPV infection at diagnosis increased with the severity of cervical neoplasia.

**CONCLUSIONS** In summary, because the pre-diagnostic viral loads among the new SIL cases, particularly HSIL, were much lower than that at diagnosis, HCT was not sensitive in detecting HPV DNA at enrollment. However, the sensitivity of HCT markedly increased at the time of diagnosis of SIL, suggesting that viral loads were higher by the time of diagnosis.

Our results suggest that the sensitivity of PCR makes the technique superior to HCT in clinical settings that require sensitive detection of all HPV infections in order to gain strong reassurance that SIL (especially HSIL) is not present nor incipient in women with normal cytology. For example, in postmenopausal women, HPV prevalence is typically low and the infections are more likely to represent worrisome (i.e. not transient) SIL. A sensitive test like PCR will reduce the chance of overlooking underlying serious SIL in postmenopausal women. However, in young, sexually active women, in whom transient HPV infection is common, the extremely high sensitivity of PCR may result in clinical non-specificity. Therefore, HCT may be more specific as a supplement to the Pap smear. In particular, HCT might be useful in colposcopy triage of young women with concurrent equivocal cytology, because the specificity of HCT favors the detection of the higher viral load infections which are clinically significant, reflecting a diagnosable SIL.

Digene Corporation has recently modified HCT by introducing a new microplate format (HC II) that lowers the HPV detection threshold from the original 10 pg/ml of HCT to 1 pg/ml or less. Preliminary analysis from the data of a study consisting of 209 women tested by HCT, HC II, and PCR show that the clinical sensitivity of the HC II with 1 pg/ml cut-point approached that of PCR with MY09 and MY11 primers (11). In addition, a PCR reverse dot blot kit has recently been developed by Roche Molecular Systems to speed and gener-

alize the MY09/MY11 system (12-13). Additional epidemiologic and clinical studies should be conducted to assess the performance of these modified techniques to guide their introduction into routine clinical settings.

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