

## Comparison of the Hybrid Capture Tube Test and PCR for Detection of Human Papillomavirus DNA in Cervical Specimens

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**The strong association of human papillomavirus (HPV) and cervical cancer makes it important to study HPV detection methods that may play a role in cervical cancer screening. We compared two DNA methods that are commonly used for HPV research in the United States: the MY09/MY11 L1 consensus primer PCR-based test and the first-generation Hybrid Capture tube method (HCT). Laboratory assays by each method were performed with 596 cervicovaginal specimens collected from participants in a large cohort study conducted in Portland, Ore. Included were 499 specimens from women whose cytology was normal and 97 specimens from women with squamous intraepithelial lesions (SILs). The overall HPV DNA positivity for known types was 22.5% by PCR compared to 13.6% by HCT. When the analysis was restricted to the 14 HPV types detectable by both methods, the sensitivity of HCT, with PCR used as the standard for HPV status, was higher for specimens from women with concurrent SILs (81.0%) than for specimens from women with normal cytology (46.7%). Among specimens testing positive by both methods, 97.2% of the time the two methods agreed on whether specimens were positive for cancer-associated HPV types. Both of these HPV test methods provide information that supplements the information provided by the Pap smear. The PCR method has higher analytic sensitivity than HCT in detecting HPV, but HCT may be helpful in identifying women with concurrent SILs.**

Research over the past two decades has convincingly demonstrated that human papillomaviruses (HPVs) are etiologically related to the development of most cases of cervical cancer (4, 5, 11, 16, 19). There has been a strong and consistent association between cancer-associated HPVs and all grades of cervical neoplasia, including low-grade squamous intraepithelial lesions (LSILs), high-grade squamous intraepithelial lesions (HSILs), and carcinoma (13, 16, 22). More than 90% of invasive cervical cancer specimens contain HPV DNA (4). These findings have important implications for the prevention of cervical cancer, a tumor that accounts for 12% of all female tumors worldwide, with a worldwide incidence of more than 400,000 cases yearly (17).

It has been proposed that HPV DNA testing be used to supplement and clarify equivocal Pap smear screening results (6, 7, 18, 24, 25). Incorporation of HPV DNA tests into screening programs might identify women at high risk for developing invasive cervical cancer and permit less aggressive management of women with mild or equivocal cytological abnormalities that are unlikely to progress due to the absence of cancer-associated HPV types.

Several HPV DNA test methods have been used in research. However, before any of these methods can be incorporated into screening programs, their sensitivity, specificity, and predictive values must be established within different populations.

Assays currently used to detect HPV DNA in cervicovaginal

specimens can be classified into three broad categories: DNA amplification methods (including various PCR-based assays), signal-amplified tests (such as the Hybrid Capture tube [HCT] test), and nonamplified methods (Southern blotting and dot blotting). DNA amplification methods are generally more sensitive than signal-amplified tests and nonamplified tests (28). However, greater sensitivity might be undesirable for some clinical applications, because of the high prevalence of self-limited HPV infection among young, sexually active females.

Thus, practical comparisons with realistic clinical populations are valuable. The two HPV DNA test methods in widest use for research purposes are the MY09/MY11 L1 consensus primer PCR-based test and the liquid RNA-DNA hybridization HCT method. A version of the HCT, from Digene Corporation (Silver Spring, Md.), has already been approved for use by the U.S. Food and Drug Administration. The intra- and interlaboratory reliabilities of each of these testing strategies are good (10, 15, 20, 22, 23). Only a few studies have directly compared the test results obtained with clinical specimens (8, 26). Our aim was to determine the relative performances of HCT and PCR with a large number of specimens from a clinical setting.

### MATERIALS AND METHODS

Cervicovaginal lavage specimens were collected from women enrolled in a large cohort study of HPV and cervical neoplasia conducted at Kaiser Permanente clinics in Portland, Ore. Details of this cohort study have been described previously (9, 21). Nearly 24,000 women were enrolled during 1989 and 1990, and most were followed for several years. At enrollment and at selected follow-up visits, Pap smears and cervicovaginal lavage samples were obtained and were handled as described previously (9).

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Each 10-ml lavage specimen was homogenized by gentle rocking, and aliquots were prepared for future use. From each specimen a 1-ml aliquot was stored at  $-70^{\circ}\text{C}$ , and the remaining 9 ml was split and centrifuged into two 4.5-ml cell pellets that were also stored at  $-70^{\circ}\text{C}$ . Pap smears and biopsy specimens were reviewed by a team of expert pathologists (D.R.S., M.E.S., and R.J.K.), using The Bethesda System (14).

Cohort participants were involved in one or more studies. Some studies used the PCR assay and others used the HCT method. From the more than 20,000 HPV tests that were performed, 599 specimens were tested for HPV DNA by both methods. Three specimens had insufficient material for testing. The 596 specimens in our analysis were obtained from 393 women; for 193 women two specimens were assayed (mean time of 33 months between lavage collections), and for 5 women three specimens were assayed (mean time of 24 months between each set of lavage collections). Subjects were 16 to 77 years of age (median age, 31 years) at the time of enrollment in the cohort study. Four hundred ninety-nine specimens were obtained from women who had normal cytology at the time of the lavage collection, and 97 specimens were from women with concurrently abnormal cytology. Expert review and confirmation of squamous intraepithelial lesion (SIL) cytopathology was achieved for 64 (66.0%) of the 97 specimens from women with abnormal cytology who were enrolled in a nested case-control study of incident SILs previously conducted within our cohort population. Details regarding this expert review process have been described previously (29). For the remaining specimens, collected from women not selected for our nested case-control study, original cytology results were used. Tests were performed without knowledge of the cytologic diagnosis and other clinical data.

Specimens were tested for HPV DNA by L1 consensus primer PCR with the MY09/MY11 primers and by HCT as described previously (2, 3, 9, 23). Tests were performed without knowledge of the cytologic diagnosis and other clinical data. The 1-ml aliquot of the original lavage was used for PCR testing, while an aliquot obtained from the 4.5-ml pellet of the same lavage sample was used for the HCT. The PCR 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 (PAP238A), PAP155, PAP291, and W13B. Specimens positive for HPV with the generic probe but negative with type-specific probes were classified as HPV DNA negative for purposes of the analyses ( $n = 55$ ) (Table 1). The HCT method identified 16 different types: 6/11, 16, 18, 31, 33, 35, 39, 42, 43, 44, 45, 51, 52, 56, and 58. Only the 14 HPV types (6/11, 16, 18, 31, 33, 35, 39, 42, 45, 51, 52, 56, and 58) detectable by design by both assay systems were considered when analyses comparing the two test methods were performed (group 1, Table 1). Thus, specimens that tested positive only for an HPV type undetectable by one of the assays were considered negative in the comparisons. To ensure that this approach did not alter our results, we repeated the analyses with inclusion of all detectable HPV types by either method (considering groups 1 and 2 in Table 1 as positive). These analyses did not change the overall results of the study. Also, results were similar when the analysis was restricted to a single specimen from each woman. Therefore, the results for all 596 specimens were included in the final analysis.

As a measure of agreement between the two methods, overall percent agreement was calculated. To account for the level of agreement expected by chance alone, percent agreement among the positive specimens and the kappa statistics were computed (1, 12). Percent agreement among positive specimens excluded all samples that were negative by both methods (chance agreement is most likely to occur when the point prevalence of infection is low). Analyses comparing agreement of overall positivity and positivity for the major cancer-associated types (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, and 58) were performed. Separate analyses were conducted for specimens from women with normal cytology and specimens from women with diagnoses of SILs. The SIL group included women with LSILs ( $n = 69$ ) and women with HSILs ( $n = 28$ ). Three specimens were collected from women with an equivocal cytologic diagnosis of LSILs. These specimens were included in the group of specimens from women with LSILs. Sensitivity was calculated for the HCT results by using the results from PCR as the reference standard because PCR is more sensitive. The sensitivities of the two diagnostic tests were compared by the standard  $Z$  test. Statistical significance was achieved when the  $P$  value of the test was less than 0.05. Analyses were conducted by Statistical Analysis System software.

## RESULTS

The two methods, HCT and L1 consensus primer PCR, were compared for their abilities to detect HPV DNA in 596 available cervicovaginal lavage specimens (Table 1). In the PCR assay, 22.5% ( $n = 134$ ) of the 596 specimens were positive for known HPV types. In the HCT assay, 13.6% ( $n = 81$ ) were positive for HPV DNA. The most frequent HPV types detected by PCR were HPV type 16 (46 specimens; 34.3% of those positive for HPV), HPV type 51 (20 specimens; 14.9%), and HPV types 31, 56, and 58 (12 specimens each; 9.0%). Similarly, HPV types detected most frequently by HCT included HPV type 16 (28 specimens; 34.6%), HPV type 51 (16

TABLE 1. Detection of HPV DNA by HCT and PCR-based methods

Group	HPV DNA detection	No. (%) of specimens in which HPV DNA was detected by the following test:	
		HCT	PCR
1	Positive for at least 1 of 14 types detectable by both test methods <sup>a</sup>	79 (13.3)	108 (18.1)
2	Positive for types detectable by only one test method <sup>b</sup>	2 (0.3)	26 (4.4)
3	HPV positive, unknown type	0	55 (9.2)
4	HPV negative	515 (86.4)	407 (68.3)
Total		596	596

<sup>a</sup> Fourteen HPV types were detectable by PCR and HCT: 6/11, 16, 18, 31, 33, 35, 39, 42, 45, 51, 52, 56, and 58.

<sup>b</sup> The types detectable by PCR only are 40, 53, 54, 55, 57, 59, 66, 68, PAP155, PAP238A, PAP291, and W13B. The HPV types detectable by the HCT method only are 43 and 44.

specimens; 19.8%), and HPV type 56 (15 specimens; 18.5%). Among samples from women with concurrent normal cytology, the HPV positivity rates for known HPV types were 12.8 and 5.4% for PCR and HCT, respectively. Among samples from women with SILs, HPV positivity rates for known HPV types were 72.2 and 55.7% for PCR and HCT, respectively.

Further analysis was restricted to the 14 types of HPV detectable by both methods. When this was done, 18.0% ( $n = 108$ ) of the 596 specimens tested positive for HPV DNA by PCR and 13.3% ( $n = 79$ ) tested positive by HCT. PCR identified as positive 36 specimens that HCT had classified as negative, while HCT identified as positive 7 specimens that were negative by PCR testing. Among the specimens obtained from women who were cytologically normal at the time of specimen collection ( $n = 499$ ), 9.0% were positive for HPV DNA by PCR and 4.2% were positive by HCT. Of the 97 specimens collected from women with confirmed SILs, 64.9% were positive by PCR and 55.7% were positive by HCT.

Overall, the two methods agreed 93% of the time on whether a specimen was positive or negative for one of the 14 HPV types detectable by both methods. The data were then analyzed by performing separate evaluations for women whose specimens were cytologically normal and women whose specimens had abnormal cytology. There was 94.4% agreement of the two tests for women with normal cytology and 84.5% agreement for the women with abnormal cytology (Table 2). The better agreement among women with normal cytology reflected the reduced prevalence of HPV in women with normal cytology compared to that in women with abnormal cytology. When samples negative for HPV DNA by both methods were excluded and the percent agreement among positive results was computed, we found the agreement between the tests to be 42.9% among women whose specimens were cytologically normal and 77.3% among women with SILs. By using PCR as the reference standard, the sensitivity of HCT was 46.7% among women with normal cytology and 81.0% among women with SILs ( $P = 0.0004$ ). Among women with SILs, the sensitivity was similar for individuals with LSILs (79.5%) and those with HSILs (84.2%) ( $P = 0.93$ ). Also, when the analysis was performed with the data stratified by age, the sensitivity of the HCT was uniformly higher among women with SILs than among those with normal cytology (data not shown). Interestingly, the sensitivity of the HCT among cytologically normal

TABLE 2. PCR and HCT results for women with normal or abnormal cytology<sup>a</sup>

Cytology and HCT result	No. of specimens		Total
	PCR negative	PCR positive	
Normal <sup>b</sup>			
Negative	450	24	474
Positive	4	21 <sup>c</sup>	25
Total	454	45	499
Abnormal <sup>d</sup>			
Negative	31	12	43
Positive	3	51 <sup>e</sup>	54
Total	34	63	97

<sup>a</sup> HPV types detected by PCR and HCT: 6/11, 16, 18, 31, 33, 35, 39, 42, 45, 51, 52, 56, 58.

<sup>b</sup> For specimens with normal cytology, the overall agreement between the assays was 94.4% and the agreement for positive specimens was 42.9%.

<sup>c</sup> Sensitivity, 46.7%.

<sup>d</sup> For specimens with abnormal cytology, the overall agreement between the tests was 84.5% and the agreement for positive specimens was 77.3%.

<sup>e</sup> Sensitivity, 81.0%.

women was highest among the youngest women. The sensitivity of the HCT was 60% among cytologically normal women 16 to 25 years of age and 30% among those 26 years of age or older ( $P = 0.09$ ).

Among samples positive by PCR for 1 of the 14 HPV types detectable by both methods, 92 (85.2%) were positive for cancer-associated HPV types (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, or 58). The similar figure for HCT was 65 (82.3%) for cancer-associated types. When specimens were classified as either positive or negative for cancer-associated HPV types and the sensitivity of the HCT assay relative to that of the PCR assay was computed, the sensitivity of the HCT was again higher among those with disease than those without disease (79.6 versus 42.1%;  $P < 0.001$ ).

Kappa statistics for overall and subgroup analyses were all in the range of 0.52 to 0.73, reflecting good agreement between the two tests.

We found good typing agreement for the 72 specimens that tested positive by both HPV DNA methods; for 58.3% of the specimens ( $n = 42$ ) the tests agreed completely with respect to the individual HPV types present in the specimen, for 34.7% of the specimens ( $n = 25$ ) the tests agreed partially (the two assays agreed on at least one HPV type), and for only 6.9% of the specimens ( $n = 5$ ) no common HPV types were detected by the two tests. The agreement of the tests was 97.2% (70 of the 72 specimens) as to whether the specimens were positive for at least one cancer-associated type.

## DISCUSSION

In concordance with previous reports, we observed that PCR-based methods for detecting HPV DNA in cervical specimens have higher sensitivities than HCT (26, 27). In addition, we noted an increased sensitivity of HCT relative to that of PCR among women with cytological abnormalities compared to that among women who are cytologically normal. Our results are consistent with those of Smits et al. (26), who reported that HCT detected 80% of 50 HPV-positive (by PCR) high-grade Pap smears, whereas HCT detected only 50% of 18 HPV-positive (by PCR) normal or inflammatory-type Pap smears. These findings may reflect an increased viral load in HPV-positive women with SILs compared to that in women who are HPV positive but cytologically normal. In fact, in our

study population HPV-positive women with abnormal cytology had significantly higher HCT values (an estimate of viral load) than HPV-positive women with normal cytology. The mean HCT levels in the two groups of women were 543.2 pg/ml (95% confidence interval = 365.7 to 720.7) and 243.9 pg/ml (95% confidence interval = 95.9 to 391.9) respectively ( $P = 0.02$ ).

Our results indicate that the PCR and HCT may have different but complementary applications in clinical settings. The HCT HPV DNA detection method may be useful when the major objective is not just to detect a viral infection but rather to detect infections that are indicative of concurrent underlying SILs, such as in young, sexually active women who characteristically have high prevalences of transient HPV infection (20). PCR-based HPV DNA detection tests may be ideal in clinical settings in which the goal is to accurately detect all HPV infections to gain reassurance that no disease is present in women with normal cytology, such as in populations of postmenopausal women in whom the HPV prevalence of HPV is typically low but who are likely to present with persistent infections. The high sensitivity of the HCT assay among subjects with SILs and the low prevalence of DNA detection among cytologically normal subjects would maximize the positive predictive value of HCT (the probability that HPV positivity indicates true underlying SILs). The high degree of sensitivity of the PCR-based assays and the low prevalence of HPV infection in older women would tend to maximize the negative predictive value of the PCR assay. Thus, the best test for HPV will vary for different clinical and research purposes.

The HCT that has been approved by the U.S. Food and Drug Administration has now been modified by Digene Corporation. The analytical sensitivity has been increased more than 10-fold (from 10 to 0.2 pg of HPV DNA per ml of processed specimen). Clinical trials of the test in a new format, the HC Microplate, are under way. The test modifications have not yet been formally analyzed. A version of the L1 consensus primer PCR test is under development at Roche Molecular Systems (Alameda, Calif.). Now that HPV DNA test development has reached a clinically useful level, the next generation of HCT and L1 PCR kits are likely to be widely used. Additional comparisons between the PCR and HCT kits will be needed to determine which techniques are best for different clinical uses.

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