

# Human Papillomavirus Detection: Verification with Cervical Cytology

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**OBJECTIVE:** Thirteen specific types of human papillomavirus (HPV), classified as high-risk for the development of cervical cancer, have been reported in 99.7% of all cervical cancers. For this reason, and because of the reported lack of sensitivity of the Papanicolaou (Pap) smear for detecting HPV, some experts believe that the use of papillomavirus DNA testing may replace cytology for routine gynecological screening. Our goal was to validate a commercial assay, the Digene Hybrid Capture-2<sup>®</sup> for the detection of human papillomavirus by comparing the results to cytological detection of cervical abnormalities.

**DESIGN:** Cytology results of concurrent liquid-based Papanicolaou smears were compared to the Hybrid Capture-2 results. Correlation was assessed and discordant cytology results were reviewed.

**SETTING:** Louisiana State University Health Sciences Center at Shreveport, Department of Pathology, HPV Diagnostic Laboratory.

**PATIENTS:** All liquid cytology specimens submitted for HPV testing between November 1, 2000 and April 1, 2001.

**RESULTS:** Of the 291 cases tested by Hybrid Capture-2, 12% and 28% were positive with the low-risk and high-risk probes, respectively, and 265 had concurrent cytology results. Fourteen specimens testing positive only with the low-risk probe were not included in this comparison. Thus, the results for 251 of the 291 (86%) specimens tested for human papillomavirus DNA were compared to the original cytology report. Overall concordance between Hybrid Capture-2 and the original smear cytology result was 78%. Slide review reduced the number of discordant specimens from 22% to 12%.

**CONCLUSION:** Based upon these data, we find the HPV assay to be useful as a routine screen for Human papillomavirus.

**ABBREVIATIONS:** ASCU = atypical squamous cells of undetermined significance; ASCUD = favor dysplasia; ASCUR = favor reactive; ASCUS = atypical squamous cells

of undetermined significance not otherwise specified; HC-2 = hybrid capture 2; HGSIL = high grade squamous intraepithelial lesion; HPV = human papillomavirus; LGSIL = low grade squamous intraepithelial lesion; Pap = Papanicolaou; WNL = within normal limits.

**INDEX TERMS:** cervical cytology; cervical dysplasia; HPV.

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Cervical cancer is a leading cause of morbidity and mortality worldwide.<sup>1</sup> Even in developed countries, despite the widespread use of the Papanicolaou (Pap) smear screening for precursor lesions, invasive cervical carcinoma remains the fourth most common malignancy among women. About 14,000 new cases of invasive cervical cancer are diagnosed per year in the U.S.; approximately 4900 women died from the disease in the U.S. in 2001.<sup>2,3</sup> The average age of newly diagnosed cervical cancer patients is 50 to 55 years; however, unlike many cancers that rarely affect young adults, this cancer often affects young women in their twenties.

Human papillomavirus (HPV) is deemed causally associated with cervical cancer.<sup>4,5</sup> Almost all cervical cancers are associated with persistent infection with certain high-risk HPV types. Thirteen specific types of HPV, classified as high-risk

for the development of cervical cancer, have been reported in 99.7% of all cervical cancers, particularly squamous cell carcinoma. For this reason, and because of the reported lack of sensitivity of the Pap smear for detecting HPV, some experts believe that the use of HPV testing may replace the Pap smear for routine gynecological screening.<sup>6-8</sup> We recently incorporated the Digene Hybrid Capture-2 (HC-2) diagnostic assay (Digene Corp, Gaithersburg MD) for the detection of HPV DNA into our anatomic pathology services. Our objective was to evaluate the efficacy of this commercially-available assay by comparing the results to cytological detection of cervical abnormalities, i.e., either the presence of HPV (koilocytosis) or cervical lesions known to be associated with HPV, in specimens collected in liquid medium (ThinPrep®, Cytoc Corp, Boxborough MA).

## MATERIALS AND METHODS

### Initial design for test use

A testing algorithm and in-service lectures including a literature review and specimen collection instructions were provided for local physicians and staff currently using the PreservCyt ThinPrep® medium (Cytoc Corporation Boxborough MA) for Pap smear collection.

### HPV testing patient cohort

HPV testing was performed on all specimens (n = 291) with sufficient quantity (4 mL fluid) submitted to the HPV Diagnostic Laboratory between 1 November 2000 and 1 April 2001, using HC-2. Both probes (high-risk and low-risk) were used. The average age for all women tested was 39 years; the mean for women testing positive for high-risk HPV was 34 years.

### Digene HC-2 method

The HC-2 technique is based upon signal amplification technology. Specimens are prepared by centrifugation of 4 mL of ThinPrep fluid into a cell pellet. The supernatant is discarded. Denaturation reagent is added to the cell pellet and incubated at 65 °C, generating single-stranded DNA. For each patient, two samples are assayed. To one sample, a low-risk probe cocktail containing five RNA probes, specific for low-risk HPV types (HPV 6, 11, 42, 43, and 44) is added. To the other sample, a high-risk probe cocktail containing thirteen RNA probes, specific for high-risk HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) is used. If HPV is present in the sample, the specific RNA probe for that type will bind to its single-stranded DNA target and form RNA-DNA hybrids. These hybrids are captured onto a solid support using antibodies that bind (nonspecifically) to RNA-DNA hybrid molecules. After

washing, enzyme-labeled anti-hybrid antibodies are added to the mixture, followed by substrate to generate a chemiluminescent signal that is read on a luminometer.

### Cytology

Gynecological smears corresponding to HC-2 samples or, if no cytology for that sample was available, corresponding to samples taken within one month before or after the time of HPV sampling, were available for 261 women. Women testing positive for the low-risk HPV probe, but negative for the high-risk probe (n = 15) were excluded. Thus, cytology for 251 women was compared to HPV status. Cytology results were classified into two categories: 1) negative, i.e. within normal limits (WNL), or atypical squamous cells of undetermined significance, favor reactive (ASCUR), or, 2) positive, i.e. not otherwise specified (ASCUS), ASCUS, favor dysplasia (ASCUD), low grade squamous intraepithelial lesion (LGSIL), HPV changes or mild dysplasia, and high grade squamous intraepithelial lesion (HGSIL): moderate, severe dysplasia or carcinoma in situ. No cases of carcinoma were found in these women.

### Correlation analysis

All laboratory results and demographic data were tabulated manually from laboratory logs and request forms, respectively. Original cytology results of concurrent (collected at the same time or within several weeks of the HPV specimen) Pap smears were compared to the HC-2 results. Concordance between HPV testing and cytology is defined either as negative HC-2 test with negative cytology (WNL or ASCUR) or as positive HC-2 with positive cytology (ASCUS, ASCUD, LGSIL, or HGSIL). Discordance is defined either as negative HC-2 with positive cytology or as positive HC-2 with negative cytology. Correlation was assessed and all discordant cases were reviewed independently by a cytotechnologist and a cytopathologist using the minor criteria for the specific diagnosis of HPV, e.g., binucleation and dyskeratosis as described.<sup>9</sup> Disagreement between cytology reviews was resolved by re-review by two of the authors working together. All cytology results are thus classified as 'original' or 'reviewed'.

## RESULTS

### HPV test results

As shown in Figure 1, during this time period, 291 patients were tested for HPV: 66% were negative for both probes, 8% were positive for both probes, 5% were positive for low-risk only, and 21% were positive for high-risk only. Overall, 36 (12%) and 82 (28%) were HPV positive with the low-risk and high-risk probes respectively.

**Cohort comparison**

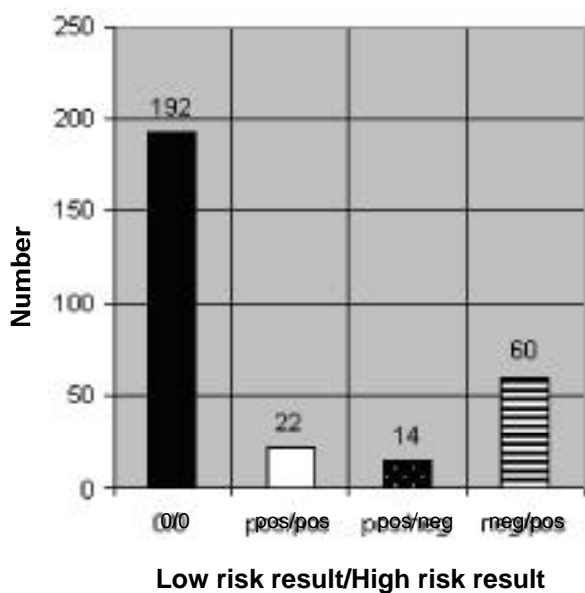
Concurrent cytology results were available for 265 specimens. However, since we are most concerned with the high-risk HPV results, specimens testing positive only with the low risk probe (n = 14), were not included in this comparison. This left a cohort of 251 patient samples for comparison study. As shown in Figure 2, of these 251 samples, 181 (72%) were negative for HPV. Thirty-three of these HPV-negative samples had positive original cytology reports (23 were ASCUS/ASCUD; 10 were LGSIL) for an original discordance of 33/181 or 18.2% of all HPV negative women. After review of the cytology smears, 22 (12%) remained discordant. Seventy samples or 28% of the total number compared tested positive for high-risk HPV. Of these, 22 had negative (normal) original cytology reports for a discordance of 31% of all HPV-positive women. After review of these cytology smears, only 9 (13%) remained discrepant. Overall, cyto-

logic evaluation alone noted koilocytosis or HPV changes in only 24% of the specimens testing positive by HC-2 assay (data not shown).

**Overall concordance**

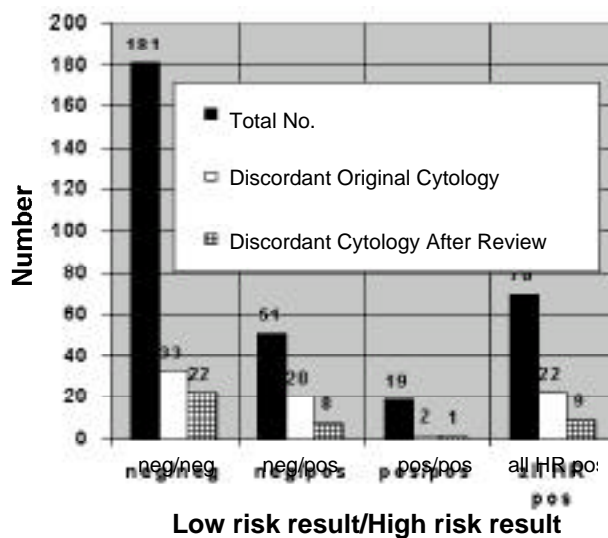
Overall concordance between HC-2 and the original Pap smear cytology result was 196/251 or 78%; overall discordance was 55/251 or 22%. Discrepant samples were submitted for slide review by both a cytopathologist and a cytotechnologist. Slide review using the minor criteria reduced the number of discordant specimens to 31/251 or 12%. Thus, overall concordance between HC-2 and Pap smear reviewed cytology was 88%. These discordant data are given in Figure 2.

**Figure 1.** Results for all HPV testing performed during the study period



Total HPV results for all 291 patients are categorized according to the number testing negative for both probes (0/0), positive for both probes (pos/pos), positive for low-risk HPV with a negative high-risk HPV test (pos/neg), and negative for low-risk HPV with a positive high-risk HPV test (neg/pos). The total number of specimens for each result are given at the top of each column.

**Figure 2.** Cytology results compared to HPV results for 251 specimens



Cytology results were compared to HPV results for 251 specimens. HPV results are categorized into three groups, negative for both probes, negative for low-risk HPV with positive high-risk assay, positive for both probes, and a combination of the second and third groups, wherein all specimens positive for high-risk HPV were included. Patients testing positive only for low-risk HPV were excluded from this analysis. The total number of specimens is shown in black, the number for which discordant original cytology was reported is shown in white. The final (checked) bar represents the number of discordant samples after review of the original slide using minor criteria.

## DISCUSSION

There is much discussion about the benefits of HPV testing and its potential as a primary screen.<sup>7,8,10,11</sup> We began this study as part of an in-house validation for the HC-2 HPV DNA assay, offering it to physicians as an alternative to immediate colposcopy or repeat cytology for patients with ASCUS Pap smears. However, judging from these cytology review data (only 12% of the specimen results remained discordant after review by a cytopathologist incorporating the minor criteria for HPV diagnosis), we believe the HPV DNA test should be incorporated into routine screening for gynecologic abnormalities.

Sellers recently predicted that if incorporated into cervical screening strategies, HPV testing has the potential advantage of increasing population coverage.<sup>12</sup> Cuzick reported that HC-2 may be a useful adjunct in that combined screening tests offer the possibility of greater protection and/or longer screening intervals which could reduce the overall cost of the screening program.<sup>5</sup> Such longer intervals would be a necessary component in such a cost-benefit program, as others do not recommend the test in countries with annual cytological screening.<sup>10</sup> Longer intervals between screening rounds is appealing. Ronco predicts this would reduce the burden for women and allow for higher coverage at each round.<sup>7</sup> He further speculates that HPV testing would segregate women at low risk for developing a lesion for several years, i.e. those who test HPV-negative, from those who should not participate in a long-interval test, i.e. those who test high-risk HPV-positive. The latter group could be followed more diligently and at shorter intervals. This certainly could apply to women over 30 in whom there is a lower prevalence of HPV.<sup>7</sup>

In conclusion, we found a 78% concordance between HPV DNA testing by HC-2 and original cytology. This is similar to that reported by others.<sup>13</sup> Discordance was reduced to only 12% after cytology review of smears. The HC-2 assay appears to have potential as an adjunct assay for cytologic screening in the prevention of cervical cancer. It bears noting that these two tests measure different endpoints. HPV DNA detection of high-risk types predicts the presence and future development of cervical intraepithelial neoplasia, whereas cytology detects cytologic abnormalities including the viral cytopathic effect of HPV.<sup>14</sup> This means that one should expect HPV positivity in women with normal cervical cytology; however, the reverse (positive cytology of LSIL or greater in women testing HPV negative) should be the exception. In our study, HC-2 missed only ten original LSIL cases and only three reviewed

LSIL cases. The use of both tests could result in the ability to use longer screening intervals for those women who test HPV-negative, with review of smears and shorter time intervals for those who test HPV-positive.

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## REFERENCES

1. Pao CC, Hor JJ, Yang F-P, and others. Detection of human papillomavirus mRNA and cervical cancer cells in peripheral blood of cervical cancer patients with metastasis. *J Clin Oncol* 1997;15:1008-12.
2. Dellas A, Schultheiss E, Leivas MR, and others. Association of p27Kip1, cyclin E and c-myc expression with progression and prognosis in HPV-positive cervical neoplasms. *Anticancer Res* 1998;18:3991-8.
3. Parkin DM, Pisani P, Ferlay J. Global cancer statistics. *Ca Cancer J Clin* 1999;49:33-64.
4. Milde-Langosch K, Riethdorf S, Loning T. Association of human papillomavirus infection with carcinoma of the cervix uteri and its precursor lesions: theoretical and practical implications. *Virchows Archiv* 2000;437:227-33.
5. Cuzick J, Beverley E, Ho L, and others. HPV testing in primary screening of older women. *Brit J Cancer* 1999;81:554-8.
6. Smith AE, Sherman ME, Scott DR, and others. Review of the Bethesda System atlas does not improve reproducibility or accuracy in the classification of atypical squamous cells of undetermined significance smears. *Cancer* 2000;90:201-6.
7. Ronco G. Use of molecular tests of human papillomavirus (HPV) as a screening test for cervix cancer: a review. *Epidemiol Prevenzione* 1999;23:372-7.
8. Kuhn L, Denny L, Pollack A, and others. Human papillomavirus DNA testing for cervical cancer screening in low-resource settings. *J Natl Cancer Inst* 2000;92:818-25.
9. Mayelo V, Garaud P, Renjard L, and others. Cell abnormalities associated with human papillomavirus-induced squamous intraepithelial cervical lesions. Multivariate data analysis. *A J Clin Pathol* 1994;101:13-8.
10. Birner P, Schindl M, Stani J, and others. Hybrid capture based human papillomavirus typing in cervical screening compared to cytology and histology. *Wiener Klin Wochenschrift* 2000;112:761-6.
11. Clavel C, Masure M, Bory JP, and others. Human papillomavirus testing in primary screening for the detection of high-grade cervical lesions: a study of 7932 women. *Brit J Cancer* 2001;84:1616-23.
12. Sellers JW, Lorincz AT, Mahony JB, and others. Comparison of self-collected vaginal, vulvar, and urine samples with physician-collected cervical samples for human papillomavirus testing to detect high-grade squamous intraepithelial lesions. *CMAJ* 2000;163:513-8.
13. Zuna RE, Moore W, Dunn ST. HPV DNA testing of the residual sample of liquid-based Pap test: utility as a quality assurance monitor. *Mod Pathol* 2001;14:147-51.
14. Ferris DG, Wright RC Jr, Litaker MS, and others. Comparison of two tests for detecting carcinogenic HPV in women with Papanicolaou smear reports of ASCUS and LSIL. *J Fam Pract* 1998;46:136-41.