A Comparison of Two Methods to Determine the Presence of High-risk HPV Cervical Infections

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Abstract and Introduction

Abstract

Clinical tests for human papillomavirus (HPV) DNA require clinical validation before being offered for use by laboratories. To determine the clinical viability of a laboratory-developed test using the Invader HPV reagents (Third Wave Technologies, Madison, WI), a retrospective study was designed using 213 patient cervical cytologic samples. The results of the Invader assay were directly compared with the results obtained using the Hybrid Capture 2 High-Risk HPV assay (Digene, Gaithersburg, MD). The results of both assays were also compared with cytologic evaluation. In addition, clinical performance was evaluated using a standard-of-care approach in which colposcopically guided biopsies were done in cases where standard of care dictated, and the histologic features of the biopsy specimens were noted. The Invader-based test demonstrated a clinical sensitivity in atypical squamous cells of undetermined significance cases of 98% for cervical intraepithelial neoplasia (CIN) 2 or worse and 100% for CIN 3 or worse and a negative predictive value of 96.9% (confidence interval, 89.3%-99.6%) using data generated mostly from the use of an earlier version of reagents. These findings support the clinical and laboratory benefits of the Invader method.

Introduction

Until recently, the only method available to screen women for cervical cancer was the use of cytology to identify morphologically abnormal cells in cellular smears. Discoveries during the last 2 decades have led to the determination that infection with certain types of the human papillomavirus (HPV) is a necessary precursor for the development of cervical cancer.[1,2] These high-risk HPV genotypes that can lead to cancer have been identified as 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68.[3-5] Consequently, the detection of high-risk HPV infection in cervical tissue in conjunction with abnormal cytologic findings represents a powerful tool that may be used to predict a woman's risk of developing cancer and has become a part of the cervical cancer screening procedure for women.[6-9]

The US Food and Drug Administration (FDA)-approved Digene HPV test (Digene, Gaithersburg, MD), which detects viral DNA, is the most widely used method to detect HPV infection in cervical tissue. Based on expansive clinical studies, professional guidelines now recommend HPV DNA testing to be performed on women with equivocal cytologic results and on women older than 30 years in conjunction with cytology as part of the routine screening process.[10,11]

Although the Digene HPV test has been shown to be an effective screening tool, it has some significant limitations. One limitation is the significant number of false-positive results (approximately 10%-19%)[12,13] owing to cross-reactivity with low-risk HPV DNA. False-positive results are defined as a positive test result in the absence of high-risk HPV DNA and a positive test result attributable to cross-reactivity with low-risk HPV DNA with the absence of high-risk HPV DNA. The former has been shown to occur almost 5% of the time[10] and the latter at least 10% of the time the Digene HPV test generates a positive result. Another is the number of inconclusive results that require retesting of the same sample or additional samples, resulting in delays and additional cost.[13] Finally, the Digene HPV test requires an increased volume of sample material (4 mL) for adequate results[14] with some sample types such as PreservCyt liquid cytology medium (Cytyc, Marlborough, MA) in comparison with other methods.[14] Volume has become important because of the desire of physicians to include other testing (eg, for Neisseria gonorrhoeae and Chlamydia trachomatis) of the Papanicolaou (Pap) specimen.
Recently, analyte-specific reagents (ASRs) have become commercially available that may be used as critical components by laboratories to develop their own tests for HPV DNA in cervical samples. The FDA policy on the use of such reagents is clear that they may be used for HPV testing.[16] The most recent guidelines for cervical cancer screening issued by professional societies also no longer recommend or require only the use of FDA-approved HPV tests.[17] The agencies that regulate clinical laboratories that use laboratory-developed tests only require analytic validation of such tests, although clinical validation is desirable.[18] Several commentators have also insisted that the only acceptable validation is an analytic and a clinical validation on the scale of the ALTS trial[19] (Atypical Squamous Cells of Undetermined Significance–Low-Grade Squamous Intraepithelial Lesion Triage Study), a study funded by the government and device manufacturers. However, for laboratories wanting to offer HPV testing using ASR-based laboratory-developed tests, a study of that size and scope is financially and technically impractical for most clinical laboratories. This study suggests it is not necessary to ensure that a laboratory-developed test be clinically validated if the test is shown to be at least equivalent to the purported "gold standard" in sensitivity and specificity because this implies the test should be clinically equivalent as well.

Third Wave Technologies (Madison, WI) has developed a set of novel molecular reagents that use the Invader chemistry to detect high-risk HPV infections in cervical cellular samples. Laboratory-developed tests using these reagents have the potential to provide results superior to those of the Digene test because they may have fewer false-positive reactions owing to the low cross-reactivity with low-risk HPV DNA imparted by the uniqueness of the Invader chemistry, may reduce the occurrence of inconclusive results leading to decreased retesting and costs, and could require as little as 2 mL of specimen for adequate test results, making sampling less of an issue.

To determine the clinical viability of a laboratory-developed test using Invader HPV reagents, a retrospective study was designed using 213 patient cervical cytologic samples. The results of the Invader assay were directly compared with the results obtained using the Digene Hybrid Capture 2 High-Risk HPV assay (HC2). The results of both assays were also compared with cytologic evaluation. In addition, clinical performance was evaluated using a standard-of-care approach in which colposcopically guided biopsies were done in cases in which standard of care dictated, and the histologic features of the biopsy specimens were noted. The laboratory performing the study is a regional reference laboratory and does not have access to biopsy results for all patients.

**Materials and Methods**

**Clinical Specimens**

A total of 5,788 specimens used in this study were cervical Pap samples collected by using the Rovers Cervex-Brush (Therapak, Oss, the Netherlands) and placed in the SurePath vial containing CytoRich preservative-fixative fluid (BD TriPath Imaging, Burlington, NC). These specimens were submitted to the cytology department at Regional Medical Laboratory, Tulsa, OK, for Pap testing. The HC2 was performed on 213 of these samples and Invader-based HPV testing was performed on all 5,788. On completion of cytologic and HPV testing, the samples were deidentified for use in the remainder of the study.

**Pap Smears and Cervical Cytology**

Cervical cytologic examination was performed using the automated PrepMate accessory and PrepStain Slide Processor (BD TriPath Imaging). After the specimen was placed on a SurePath slide and Pap stained, a glass coverslip was applied by a SurgiPath automatic coverslipper (SurgiPath, Richmond, IL). The specimen container was removed from the PrepStain instrument, and 2 mL of SurePath preservative fluid was added.

Pap smears were evaluated by using the FocalPoint (BD TriPath Imaging) automated Pap instrument. Up to 25% of the Pap smears needed no further review following the FocalPoint evaluation. The remaining cases were manually screened by a cytotecnologist using the 2001 Bethesda System.[20] Pap smears in the category of atypical, reactive, or repair were then evaluated by a board-certified cytopathologist who also interpreted the smears based on the 2001 Bethesda System.
Digene HC2 High-Risk HPV DNA Test

The HC2 test was performed as per manufacturer's instructions at Quest Diagnostics (San Juan Capistrano, CA). This is an isothermal signal-amplification method that uses crude cell lysis with denaturation of the resulting DNA before probe hybridization.

Nucleic Acid Extraction

The Invader-based method requires the use of purified extracted DNA. The extraction method is detailed subsequently and takes only slightly longer to perform than the sample processing (lysis and denaturation) for the Digene HPV test. The SurePath specimens were stored at 2°C to 6°C and processed within 30 days of collection. DNA was extracted from SurePath cervical specimens by using a modified and previously validated PureGene Prep (Gentra Systems, Minnetonka, MN) protocol. First, 2 mL of each specimen was centrifuged for 5 minutes, followed by the addition of 300 µL of Puregene Cell Lysis Solution (Gentra Systems), and incubated at 95°C for 5 minutes, followed by cooling to 50°C. This step was followed by incubation with 1.5 µL of Proteinase K at 55°C for 15 minutes. The mixture was then cooled to room temperature, and 100 µL of protein precipitation solution was added. The sample was vortexed vigorously for 20 seconds and placed at –70°C for 5 minutes. The sample was thawed and then centrifuged for 2 minutes followed by the addition of 1.5 µL of glycogen (20 mg/mL) to create a DNA pellet. Finally, 300 µL of 100% isopropanol was added followed by centrifugation for 5 minutes. The supernatant was poured off again, and the DNA was dried at 65°C for 10 minutes. The final mixture was resuspended in 100 µL of RNase- and DNase-free water and used for Invader-based testing.

Invader HPV Testing

Invader chemistry is an isothermal signal-amplification procedure that uses the formation of partially overlapping structures, followed by recognition and cleavage of the structure by a modified flap endonuclease. This results in the release of a cleaved DNA fragment that simultaneously interacts with a FRET cassette, resulting in detectable fluorescence. The procedure also is a biplex reaction containing probes for the detection of the HPV-specific targets as well as a human gene that serves as an internal control for sample DNA sufficiency and for the presence of potentially interfering substances. A graphic representation of the Invader chemistry is shown in Figure 1.

Figure 1.

Extracted DNA samples were tested for the presence of high-risk HPV by using a laboratory-developed HPV assay that uses Invader HPV ASRs. Three separate Invader reactions were performed with each reaction using 1 of 3 separate oligonucleotide pools for each sample. Two versions of the Invader high-risk HPV ASRs were used in the study, V1.0 and V2.0. The majority of the testing was performed using the V1.0 reagents with approximately 10% of the samples being tested with the V2.0 reagents, as the V1.0 reagents were discontinued by the manufacturer with the release of the V2.0 reagents.

Each version of the ASR reagents (V1.0 and V2.0) that we used consisted of 3 separate oligonucleotide pool sets with FAM (6-carboxy-fluorescein)-labeled probes, one oligonucleotide set specific for a human gene, the human α-actin gene (ACTA1) for V1.0 and the human histone 2 gene (HIST2H2BE) for V2.0, each labeled with RED (Redmond Red, Epoch Biosciences, Bothell, WA) for the internal control, Cleavase enzyme (Third Wave Technologies), and magnesium chloride solution.

The V1.0 probe pool sets that were included were pool A5/A6, containing the oligonucleotides required for the detection for HPV types 51 and 56; pool A7 for the detection of HPV types 18, 39, 45, 59, and 68; and pool A9 for the detection of HPV types 16, 31, 33, 35, 52, and 58. The V2.0 probe sets were modified so that the A5/A6 pool also contained probes to detect a 14th high-risk HPV type, HPV type 66, as recommended for all high-risk HPV DNA screening tests; probes in the A7 pool were modified to accommodate recently identified polymorphisms with the target sequences and in the A9 pool were modified to improve detection of HPV type 16. The V1.0 reagents were used to test the first 5,242 samples, and the V2.0 reagents were used to test the remaining 546 samples.

To perform the assay, the DNA samples were mixed by vortexing. Three 10-µL aliquots of each sample were added to separate wells of a 96-well reaction plate and layered with 20 µL of mineral oil. The plates were incubated at 95°C for 5 minutes in a PTC-100 Peltier Thermal Cycler (MJ Research, Waltham, MA) to denature the DNA and then cooled to 63°C. A 10-µL solution of master mix containing 1 of the 3 oligonucleotide sets, plus the internal control oligonucleotide pool, was added to each of the triplicate aliquots. Each master mix consisted of 4 µL of magnesium chloride, 4 µL of 1 FAM-labeled oligonucleotides mix, 1 µL of RED-labeled oligonucleotides mix, and 1 µL of Cleavase enzyme, per reaction. The plates were then incubated at 63°C for an additional 4 hours, after which they were cooled to ambient temperature for 5 minutes. Readings were taken immediately after cooling using a GENios microplate fluorometer (Tecan, Durham, NC). Excitation/emission settings were 485/535 and 560/612 nm for FAM and RED, respectively. Data collected from the fluorometer were imported into a generic Microsoft Excel (Microsoft, Redmond, WA) spreadsheet programmed with user-defined cutoffs and used to first determine that the sample had sufficient cellularity for analysis based on sufficient internal control RED signal and second, the positivity or negativity of the reactions for samples with sufficient cellularity.

**Invader Analyte Data Analysis**

Invader genotypes were generated using an Excel program containing user-defined criteria. Analysis consists of division of the FAM and RED signals generated from patient samples by the background fluorescence of the no-target control and the result compared with a user-defined cutoff. In addition, the RED signals generated by the 3 reactions per patient sample (the internal control signals) were compared by the spreadsheet software with flag variances between wells for a single sample, an indicator of potential pipetting errors.

**PCR With Sequencing**

Samples that yielded discordant results between the Digene and Invader tests were resolved by PCR with sequencing. Sequencing was performed by Third Wave Technologies. Each discordant sample was amplified by 2 HPV consensus primer pairs, GP5+/GP6+ and L1C1/L1C2. The amplicons were gel-purified and cloned in a high-copy vector. Two colonies were picked from each, for a total of 4 colonies per discordant sample. Plasmids were purified from these colonies and sequenced. The resulting sequences were compared with all human HPV sequences in the GenBank DNA sequence library using a BLAST analysis, and the top homology score for each sequence was listed as the genotype for that particular sample.
Histologic Results

By using a standard-of-care approach, colposcopically guided biopsies were done when the standard of care dictated, and the histologic features of the biopsy specimens were noted. Regional Medical Laboratory, the laboratory performing the study, is a regional reference laboratory and did not have access to all biopsy results for all patients undergoing colposcopy and treatment.

Results

Analytic Validation

For the first 213 samples tested, results were obtained by using the Invader V1.0 reagents and the HC2 method for 207 samples. Invader results were indeterminate for 4 samples owing to insufficient amounts of residual DNA or cells in the cytologic sample. Since HC2 does not use an internal control for sample sufficiency, it was not possible to determine if any of the HC2 HPV– results were due to insufficient DNA in the sample (hypocellularity) or whether they simply did not contain high-risk HPV DNA without performing PCR with sequencing on every sample. The HC2 results were unavailable for 2 samples owing to processing errors within the laboratory.

The Invader and HC2 testing results were in agreement for 188 patient samples. There were 19 discordant results between Invader and HC2 testing, 13 HC2+/Invader– and 6 HC2–/Invader+, 18 of which were resolved by PCR with sequencing Table 1. Using PCR with sequencing testing as the final arbiter and defining a false-positive as a positive test result with no HPV DNA detected by PCR with sequencing or a positive test result with only low-risk HPV DNA detected by PCR with sequencing, it was demonstrated that the HC2 test gave incorrect results in 12 of 18 cases (11 false-positive and 1 false-negative). The Invader V1.0 test was incorrect in 6 of 18 cases (4 false-positive and 2 false-negative). In 6 cases of the Invader and HC2 false-positive cases (3 for each HPV testing method), there was no HPV detected by PCR with sequencing.

Overall, the HC2 method displayed significantly greater cross-reactivity to low-risk HPV types than did the Invader test (3.9% vs 0.5%). The HC2 test cross-reacted to a number of low-risk HPV types: 6, 42, 52, 53, 58, 66, 69, 82, 84, and 86, whereas the Invader method displayed cross-reactivity to only 1 low-risk HPV type: 83. The false-negative rate was slightly higher by the Invader V1.0 method (0.97% vs 0.5%). In these cases, the Invader V1.0 method failed to detect HPV 16 in one sample and HPV 58/59 in the other. The HC2 method failed to detect a sample that was sequence-positive for HPV type 56 Table 2.

Comparison of Discordant Results With Cytologic Results

Comparison of discordant HPV DNA testing results with cytologic results for the first 213 samples is shown in Table 2. For the 18 discordant results, the cytologic results were as follows: atypical squamous cells of undetermined significance (ASC-US), 9 cases; low-grade squamous intraepithelial lesion (LSIL), 8 cases; and nonneoplastic, 1 case. In 6 ASC-US cases, a false-positive result was found (4 by HC2 and 2 by Invader V1.0) and 3 cases were false-negatives (2 by Invader and 1 by HC2). All 8 LSIL cases and 1 nonneoplastic case were associated with a false-positive result (7 by HC2 and 2 by Invader).

Clinical Validation

Liquid cytology–based Pap testing and Invader-based HPV testing were performed on 5,788 specimens with 5,242 specimens tested with the Invader V1.0 reagents and 546 tested with the V2.0 reagents. Of the specimens tested, 2,121 were positive for high-risk HPV DNA, 3,583 were negative for high-risk HPV DNA, 57 were indeterminate for high-risk HPV DNA, and an additional 27 specimens had low genomic DNA (human histone 2 gene), making them nonevaluable for HPV DNA. In these cases, the lack of an internal control for the HC2 test would have resulted in an HC2– result even though insufficient quantities of DNA were present to generate a valid result. The cytologic and Invader results are given in Table 3. Several patients had more than one specimen each in the database. After removal of duplicate samples, 5,761 unique samples remained, including 3,197 ASC-US samples that were used in the subsequent analyses.
The data were also examined to determine what effect the addition of probes for HPV type 66 into the A5/A6 probe pool and the modification of the A7 and A9 probe pools had on the HPV+ rates for each cytologic category. The HPV+ rate for ASC-US samples when tested with V1.0 reagents was only 37.9%, lower than what had been expected based on previously published HPV+ rates\[23\] Table 4. Although only a small number of samples were tested using the Invader V2.0 reagents, the modifications made to the probes resulted in the HPV+ rate in the ASC-US samples increasing to 46.8%, similar to that previously reported by other investigators.

Of 3,197 ASC-US samples tested, biopsy results were not available for 2,620. This was expected because the cytologic and HPV testing was performed in a regional laboratory and the histologic examination of specimens from colposcopically guided biopsies was often performed elsewhere at local pathology laboratories not associated with the regional laboratory. As a result, biopsy results were available only for 577 patients with ASC-US cytology. These included 66 with a histologic diagnosis of cervical intraepithelial neoplasia (CIN) 2 and 26 with CIN 3 Table 5. Of the biopsy specimens, 129 with normal or reactive histologic diagnoses were positive for high-risk HPV by the Invader method. A possible explanation of this finding could be that some clinicians may have overaggressively biopsied their ASC-US cases.

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The clinical sensitivity of the Invader-based high-risk HPV test for a histologic diagnosis of CIN 3 or worse was 100% (27/27) and was 98% for a histologic diagnosis of CIN 2 or worse (91/93) with a negative predictive value of 96.9% (confidence interval [CI], 89.3%-99.6%). However, it is thought that these statistics are skewed owing to the use of data only from ASC-US cases for which biopsy histologic diagnoses were available and by their high disease prevalence (16%). Colposcopy with guided biopsy or biopsies detects approximately two thirds of CIN 3+.\[24\] Assuming that one third of 2,620 patients for whom no biopsy results were available would not have disease (≥CIN 3) detected by colposcopy with biopsy and using the lower end of the calculated CI for the negative predictive value given above (~90%), which was obtained using the data from the patients for whom biopsy results were available, it may be extrapolated that an additional 40 disease-prevalent patients would be found within the 2,620 patients; of these, the Invader-based HPV test would be positive for 36 of 40 patients and negative for 4 of 40 patients. Based on these assumptions and extrapolations, the clinical performance of the Invader-based high-risk HPV test would be as follows: sensitivity, 95.49% (95% CI, 90.44%-98.33%); specificity, 63.28% (95% CI, 61.55%-64.99%); predictive value of positive test result, 10.14% (95% CI, 8.53%-11.95%); and predictive value of negative test result, 99.69% (95% CI, 99.33%-99.89%)

Discussion

The results of this study indicate that the Invader method is at least equivalent in performance to HC2 and has the potential to offer improved clinical laboratory HPV testing. For this study, Invader results were in analytic agreement with HC2 results in 90.8% of cases yet had a lower false-positive rate owing to decreased cross-reactivity with low-risk HPV. The Invader method yielded these results while using only 2 mL of sample material. This sample volume is significantly smaller than that required by the HC2 test, which requires up to 4 mL of some liquid cytology sample types such as PreservCyt medium.\[14\] These promising results have the potential to offer significant benefit to the patient and the clinical laboratory.

The current clinical usefulness assigned to HPV testing is its use in conjunction with an ASC-US cytologic result for identifying patients who should be referred for colposcopic examination of the cervix.\[17,23\] The greatest strength of HPV DNA testing is its negative predictive value for risk of high-grade cervical disease. This is driven by the clinical sensitivity and clinical specificity of the test used. Although this study did not have access to the biopsy results for the majority of patients with ASC-US cytologic findings, sufficient biopsy results were available to determine that the clinical sensitivity of the HPV DNA results generated by this laboratory-developed test using the Invader high-risk HPV ASRs is more than adequate for use in routine clinical testing for HPV DNA as an adjunct to liquid-based cytology in identifying patients in need of referral for colposcopy. The sensitivities of 98% for CIN 2 or worse and 100% for CIN 3 or worse are greater than those published for the Digene HC2 test, and the Invader method’s negative predictive value was 96.9% (CI, 89.3%-99.6%) using data generated mostly from the use of the V1.0 reagents, not the improved V2.0 reagents. This suggests that the Invader method would have at least the same clinical benefit as the Digene method.
The potential clinical benefits of the Invader method are strongly suggested in this study. The current American College of Obstetricians and Gynecologists recommendation is to perform HPV testing for patients with abnormal cytologic findings and patients older than 30 years.[25] For patients with ASC-US or LSIL, a positive HPV test triggers repeated testing in 3 to 6 months or colposcopy.[26-28] The HC2 is known to have a significant rate of false-positive reactions owing to cross-reactivity with low-risk HPV types and generation of a positive result when no HPV DNA is present (approximately 10%-19%).[12-14] The clinical significance of this finding is supported by the latest professional guidelines for the management of women with abnormal cervical cancer screening test results from the American Society for Colposcopy and Cervical Pathology.[17] These evidence-based guidelines maintain that testing for low-risk (nononcogenic) HPV types has no role in the evaluation of women with abnormal cervical cytologic results. In fact, the latest guidelines specifically state, “There is no clinical utility in testing for other (nononcogenic) types. Testing for other (nononcogenic) HPV types when screening for cervical neoplasia, or during the management and follow-up of women with abnormal cervical cytology or cervical neoplasia, is unacceptable.”[17] In this study and based on the aforementioned guidelines, in just the first 213 samples tested, the HC2 test could have resulted in 11 potentially needless colposcopy procedures compared with 3 using the Invader method. This potential clinical advantage may offer a psychological advantage to patients while lowering medical care costs and morbidity and mortality associated with biopsy.

The Invader method could potentially provide benefits to the clinical laboratory that are not provided by HC2. A number of studies have shown significant analytic inaccuracy and reproducibility problems of HC2 testing when results are near the 1.0 positive-negative cutoff zone.[28] Consequently, a number of clinical laboratories perform a retest or request another sample when HC2 results are near this cutoff, resulting in a "gray zone." The Invader method, owing to its high degree of specificity, has no gray zone and potentially leads to less sample retesting and, thus, lowers costs and provides a better turnaround time. Also, owing to the strengths of the Invader method, less sample material is required as compared with the HC2 test (2 vs 4 mL for some sample types), reducing the percentage of quantity-not-sufficient samples frequently seen when testing using HC2. Consequently, Invader-based testing generally provides a residual sample that may also be available for confirmatory testing or for other esoteric testing (eg, PCR sequencing) or further routine testing for other organisms (eg, *N gonorrhoeae* or *C trachomatis*) that may become standard in the future. Finally, the Invader method contains an internal control to determine the presence of sufficient DNA for reliable results and for the presence of potentially interfering substances. The HC2 test does not contain this control; therefore, laboratory professionals will never know if a negative HC2 result is due to the absence of HPV DNA or a simple lack of cellularity on the liquid cytology sample. This might affect how a clinician approaches the question of resampling vs wait and follow-up vs biopsy.

In this study, the Invader method had a slightly increased false-negative rate when compared with the HC2 test (0.97% vs 0.5%) when the Invader V1.0 reagents were used. However, owing to the small study number, it is impossible to determine whether this trend is real. More recently, the Invader probe cocktail has been revised to increase the capture rate for HPV high-risk types 16, 58, and 59, which should result in decreasing the false-negative rate. A larger study is required to determine the actual false-negative rate of the Invader method using the V2.0 reagents. An additional change in the Invader probes is the addition of probes for HPV type 66, recently classified as a high-risk HPV type and that has been recommended for inclusion in all HPV DNA screening tests.[5,19]

**Conclusions**

As part of an ongoing clinical validation of a laboratory-developed test using ASR products for HPV DNA testing, this study has demonstrated that studies approaching the size and expense of the ALTS trial are not required to validate laboratory-developed HPV tests, especially when the sensitivity and specificity are at least equivalent. It must also be remembered that in any screening test, the negative predictive value is most important. This pilot study demonstrates the potential clinical and laboratory benefits of the Invader method. These benefits include more accurate testing, fewer unnecessary procedures for the patient (not to mention less psychological stress), and less laboratory retesting. This ultimately may result in better patient care and cost savings to patients and third-party payers.
**Table 1. Agreement Between Invader and HC2 Results**

<table>
<thead>
<tr>
<th>Concordant Samples</th>
<th>Discordant Samples</th>
<th>Discordance Resolved With PCR/Sequencing</th>
<th>Sequence in Agreement With Invader</th>
<th>Sequence in Agreement With HC2</th>
</tr>
</thead>
<tbody>
<tr>
<td>198/207 (90.8%)</td>
<td>19/207 (9.2%)</td>
<td>18/19 (95%)</td>
<td>12/18 (67%)</td>
<td>6/18 (33%)</td>
</tr>
</tbody>
</table>

HC2, Hybrid Capture 2; PCR, polymerase chain reaction.

**Table 2. False-Positive and False-Negative Results of Invader and HC2 Compared With Cytologic Results***

<table>
<thead>
<tr>
<th>Cytology Results</th>
<th>Discrepancies</th>
<th>Invader</th>
<th>HC2</th>
<th>Invader</th>
<th>HC2</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASC-US</td>
<td>9</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>LSIL</td>
<td>8</td>
<td>1</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nonneoplastic</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total (rate for 207 samples)</td>
<td>18</td>
<td>4 (1.9%)</td>
<td>11 (5.3%)</td>
<td>2 (1.0%)</td>
<td>1 (0.5%)</td>
</tr>
</tbody>
</table>

ASC-US, atypical squamous cells of undetermined significance; HC2, Hybrid Capture 2; HPV, human papillomavirus; LSIL, low-grade squamous intraepithelial lesion; PCR, polymerase chain reaction.

*False-positive is a positive test result with no HPV DNA detected by PCR with sequencing or a positive test result with only low-risk HPV DNA detected by PCR with sequencing. False-negative is a negative test result with high-risk HPV DNA detected by PCR with sequencing.

**Table 3. Invader HPV Result vs Cytologic Result***

<table>
<thead>
<tr>
<th>Cytologic Result</th>
<th>Positive</th>
<th>Negative</th>
<th>Indeterminate</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonneoplastic</td>
<td>177</td>
<td>1,321</td>
<td>27</td>
<td>1,525</td>
</tr>
<tr>
<td>Atypical glandular cells</td>
<td>15</td>
<td>58</td>
<td>2</td>
<td>75</td>
</tr>
<tr>
<td>Atypical endocervical cells</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>ASC-US</td>
<td>1,245</td>
<td>1,945</td>
<td>25</td>
<td>3,215</td>
</tr>
<tr>
<td>ASC-H</td>
<td>54</td>
<td>44</td>
<td>0</td>
<td>98</td>
</tr>
<tr>
<td>LSIL</td>
<td>525</td>
<td>198</td>
<td>2</td>
<td>725</td>
</tr>
<tr>
<td>HSIL</td>
<td>105</td>
<td>17</td>
<td>0</td>
<td>122</td>
</tr>
</tbody>
</table>

ASC-H, atypical squamous cells, cannot exclude HSIL; ASC-US, atypical squamous cells of undetermined significance; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion.

* For all samples collected.
### Table 4. Invader HPV V1.0 and V2.0 Results vs Cytologic Results*

<table>
<thead>
<tr>
<th>Cytologic Result</th>
<th>Invader V1.0</th>
<th>Invader V2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonneoplastic</td>
<td>11.3 (165/1,356)</td>
<td>7 (12/169)</td>
</tr>
<tr>
<td>ASC-US</td>
<td>37.9 (1,197/2,922)</td>
<td>40.8 (137/329)</td>
</tr>
<tr>
<td>ASC-H</td>
<td>54 (49/91)</td>
<td>71 (5/7)</td>
</tr>
<tr>
<td>LSIL</td>
<td>70.6 (482/683)</td>
<td>88 (53/60)</td>
</tr>
<tr>
<td>HSIL</td>
<td>88.3 (98/111)</td>
<td>64 (7/11)</td>
</tr>
</tbody>
</table>

ASC-H, atypical squamous cells, cannot exclude HSIL; ASC-US, atypical squamous cells of undetermined significance; HPV, human papillomavirus; HR, high-risk; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion.

* Rates are given as percentage (number positive/number tested). V1.0 reagents used ACTA1 as an internal control and probes for 13 HR HPV types. V1.0 reagents were discontinued by the manufacturer during the study. V2.0 reagents used HISTH2BE as an internal control and probes for 14 HR HPV types, including type 68.

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### Table 5. Invader HPV vs Histologic Result for 577 ASC-US Samples

<table>
<thead>
<tr>
<th>Histologic Result</th>
<th>Positive</th>
<th>Negative</th>
<th>Indeterminate</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonneoplastic/reactive</td>
<td>129</td>
<td>44</td>
<td>1</td>
<td>174</td>
</tr>
<tr>
<td>CIN 1</td>
<td>289</td>
<td>19</td>
<td>2</td>
<td>310</td>
</tr>
<tr>
<td>CIN 2</td>
<td>64</td>
<td>2</td>
<td>0</td>
<td>66</td>
</tr>
<tr>
<td>CIN 3</td>
<td>26</td>
<td>0</td>
<td>0</td>
<td>26</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus.

Source: Am J Clin Pathol © 2008 American Society for Clinical Pathology
References


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