Assessment of Fluorescence in Situ Hybridization and Hybrid Capture 2 Analyses of Cervical Cytology Specimens Diagnosed as Low Grade Squamous Intraepithelial Lesion for the Detection of High Grade Cervical Intraepithelial Neoplasia

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OBJECTIVE: To assess Hybrid Capture 2 (HC2) and fluorescence in situ hybridization (FISH) for the detection of cervical intraepithelial neoplasia 2 or worse (CIN 2+) in patients with a cytologic diagnosis of low grade squamous intraepithelial lesion (LSIL).

STUDY DESIGN: Residual samples from 115 LSIL-diagnosed cervical cytology specimens were evaluated by high-risk human papillomavirus (HR-HPV) HC2 testing and FISH using biotin-labeled probes to HR-HPV and chromosomal probes to 3q26 (TERC) and 8q24 (CMYC). A cervical biopsy diagnosis of CIN 2+ was considered as evidence of high grade disease.

RESULTS: The positive and negative predictive values of HC2 and FISH for detecting patients with CIN 2+ were 32% vs. 37% and 100% vs. 93%, respectively. The sensitivities of HC2 and FISH for CIN 2+ were not significantly different (100% vs. 90%, p = 0.25), while the specificity of HC2 was significantly lower than that of FISH.

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0884-6812/10/3203-0121/$18.00/0 © Science Printers and Publishers, Inc.

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FISH (28% vs. 48%, p = 0.003). FISH diagnosed fewer specimens as positive as compared to HC2 (62% vs. 79%).

CONCLUSION: These preliminary data suggest that FISH testing may be useful for determining which patients with LSIL are most likely to have CIN 2+ on clinical follow-up. (Anal Quant Cytol Histol 2010;32:121–130)

Keywords: biomarkers; cervical diseases; in situ hybridization, fluorescence; Hybrid Capture.

Two clinically challenging diagnostic categories in cervical cytology are atypical squamous cells of undetermined significance (ASC-US) and low grade squamous intraepithelial lesion (LSIL), which account for roughly 85% of all abnormal cervical diagnoses.1 Approximately 68–85% of ASC-US and 61–91% of LSIL will have negative clinical follow-up within 3 years when left untreated.2-4 However, 8–20% of ASC-US and 10–30% of LSIL will have high grade squamous intraepithelial lesion (HSIL) upon colposcopy-directed biopsy.5 In addition, approximately 65% of patients with a follow-up histologic high grade cervical intraepithelial neoplasia (CIN) were originally diagnosed with ASC-US or LSIL on a previous cervical smear.1

Human papillomavirus (HPV) is one of the major etiologic factors contributing to the development of cervical carcinoma.6-9 There is extensive evidence that HPV testing by Hybrid Capture 2 (HC2) (QIAGEN, Inc., Valencia, California, U.S.A.) on cervical cytology specimens is a valuable resource for triaging patients with an ASC-US diagnosis to colposcopy and also for primary cervical screening of women over the age of 30.10,11 Patient management guidelines defined by the American Society of Colposcopy and Cervical Pathology recommend that patients with a diagnosis of ASC-US undergo high-risk human papillomavirus (HR-HPV) testing, and those with a positive HPV result are triaged to colposcopy.11 There is currently no effective triage method for most women (excluding adolescents and postmenopausal women) with a cervical cytology diagnosis of LSIL. These women are recommended to undergo colposcopy to identify more serious cervical lesions.11 However, only 10–30% of these women will have, or progress to, high grade dysplasia, causing many of these patients to undergo unnecessary treatment. This may cause inconsistency in the follow-up management of patients diagnosed with LSIL.12,13 Some clinicians appear to prefer a “sit and wait” approach to allow time for the lesion to spontaneously regress, while others favor more aggressive clinical management.12,13 This underscores the need for tests that can effectively identify which patients are at greatest risk of developing high grade dysplasia.

Fluorescence in situ hybridization (FISH) is a technique that uses fluorescently labeled DNA probes to evaluate cells for chromosomal alterations.14 FISH has been shown to be useful in detecting genomic abnormalities in cytologic specimens of cervical dysplasia.15-20 A recent study suggested that a FISH probe set containing probes to HR-HPV has sensitivity and specificity for HPV detection similar to those of HC2 and polymerase chain reaction (PCR).21 However, there are no known studies to date that compared the diagnostic capabilities of FISH vs. HC2 for the detection of high grade disease in patients with cervical cytology specimens interpreted as LSIL. The ability of FISH to detect chromosomal alterations and HR-HPV on monolayer cervical cytology slides may improve the ability to identify which patients with an LSIL diagnosis are most likely to have high grade disease. In this feasibility study, we assessed the utility of a multiprobe FISH assay to detect patients at highest risk of having or progressing to high grade dysplasia after a cervical cytology diagnosis of LSIL.

Methods

Patient Population

After approval by the Mayo Clinic Institutional Re-
view Board, a total of 204 previously collected, residual cervical cytology ThinPrep (Hologic, Marlborough, Massachusetts, U.S.A.) vials were randomly selected for research testing from women who underwent a same-day colposcopy-directed biopsy or had a follow-up biopsy within 1 year of the cytology specimen. When a patient had > 1 follow-up histology specimen, the most severe histology diagnosis was used as the gold standard. Patient ages range from 18 to 73 years, with a median age of 24 and a mean of 29. The cytology slides were originally screened and dotted by a trained cytotechnologist, and a final diagnosis was determined by a pathologist. The colposcopy-directed biopsies were also evaluated by the pathologist as part of clinical practice. For quality control purposes, all cytology specimens were reviewed by 2 pathologists without knowledge of the previous pathologic results or patient clinical history, and a final consensus diagnosis was rendered. The retrospective review diagnoses of the cervical cytology specimens were classified as negative for intraepithelial lesion (n = 28), ASC-US (n = 33), ASC-US cannot rule out a high grade lesion (n = 2), low grade intraepithelial lesion (n = 115), high grade intraepithelial lesion/moderate dysplasia (HSIL) (n = 13) and HSIL/severe dysplasia or worse (n = 13).

The 115 specimens diagnosed as LSIL on retrospective review were chosen for additional HC2 and FISH analysis for this study. All follow-up histology slides were reviewed by 2 pathologists, without knowledge of specimen diagnoses or patient clinical history, and a consensus diagnosis was determined. The final histology diagnosis of these 115 patients included 38 (33%) negative, 48 (42%) with CIN grade 1 (CIN 1), and 29 (25%) with CIN grade 2 or worse (CIN 2+; includes 26 patients as CIN 2 and 3 patients as CIN 3).

Cytology Specimen Preparation

Cervical cytology specimens were prepared for clinical testing on the same day that they were received in the laboratory with a T2000 ThinPrep processor (Hologic). A 5-mL aliquot of the residual cytology specimen in PreservCyt (Hologic) medium was then sent for HR-HPV testing by HC2. HC2 testing was performed using the U.S. Food and Drug Administration–approved package insert method, which uses a test cutoff value of ≥ 1 pg/mL of HR-HPV DNA. The remaining cytology material was processed to produce a single ThinPrep slide for FISH analysis. The FISH slides were immersed in 2× saline/sodium citrate (SSC) at 73°C for 2 minutes, pepsin (0.5 mg/mL in 10 mM HCL) at 37°C for 10 minutes, phosphate buffered saline (PBS) at room temperature for 5 minutes, 1% formaldehyde working solution at room temperature for 5 minutes and PBS at room temperature for 5 minutes. The slides were dehydrated by placing in 70%, 85% and 100% ethanol for 1 minute each, and the slides were then air dried.

FISH Preparation

The cervical probe set consisted of biotin labeled HR-HPV probe cocktail (types 16, 18, 30, 45, 51 and 58) and directly labeled locus-specific indicator (LSI) probes to 3q26 (TERC) and 8q24 (CMYC). The LSI probes were labeled with SpectrumGold (3q26) (Abbott Molecular Inc., Des Plaines, Illinois, U.S.A.) and SpectrumRed (8q24) (Abbott Molecular) fluorophores. FISH was performed by placing 10 μL of probe on the slide, putting a coverslip over the probe, sealing the edges of the coverslip with rubber cement and then placing the slide on a HyBrite (Abbott Molecular) instrument. The probe and cellular DNA were codenatured at 72°C for 2 minutes and allowed to anneal overnight at 37°C. The slide was washed the following morning in 2× SSC/0.1% NP-40 at 48°C for 2 minutes and then rinsed in 2× SSC/0.1% NP-40 for 1 minute at room temperature. The slides were treated with an Alexa Fluor 488 Tyramide amplification kit (Molecular Probes Inc., Eugene, Oregon, U.S.A.) to develop the fluorescent signal of the HPV probe as previously described and was visualized by using a SpectrumGreen filter (Abbott Molecular). Ten microliters of DAPI I (4′,6-diamidino-2-phenylindole dihydrochloride) counterstain was applied, and the slides were coverslipped.

Analysis of Cytology Specimens by FISH

All slides were evaluated without knowledge of patient clinical history or cytology and surgical pathology diagnoses. A cytotechnologist used an Olympus BX61 fluorescence microscope (Olympus America Inc., Center Valley, Pennsylvania, U.S.A.) to manually scan the slide with a 40× oil-immersion objective, ensuring adequate overlap of each field of view. The entire area of the specimen was evaluated by using the DAPI and/or dual red-green filters to scan for morphologically atypical and/or HPV infected cells. Atypical morphologic features included nuclear enlargement, irregular nuclear contour and mottled nuclear DAPI staining. Physical
HPV status (i.e., integrated vs. episomal) and the number of 3q26 and 8q24 signals in these cells were documented. Cells were considered to have an episomal HPV infection when a uniform HPV signal was observed (contained within and covering the entire nucleus), whereas those with an integrated HPV infection were seen as a punctate HPV signal pattern (seen as 1 or more condensed dots within the nucleus). Scanning with the dual red-green filter allowed visualization of the HPV status of cells (seen as green) as well as the enumeration of the 8q24 signals in each cell (seen as red signals). The background autofluorescence of the dual red-green filter also allowed general observation of morphologic characteristics of cells such as nuclear size and nuclear border irregularity. The gold filter was used to enumerate the 3q26 signals. The scanning method was used because atypical cervical cells were often isolated among large numbers of normal epithelial cells. We have observed that the majority

![Representative FISH images (×1,000). (A) A cell with episomal HPV infection (showing a diffuse green signal pattern) and cells negative for HPV. (B) A cell exhibiting integrated HPV infection (shown as a punctate signal pattern). (C) Composite image of an abnormal cell exhibiting chromosomal gains (4 gold probes (3q26 [TERC]) and 3 red probes (8q24 [CMYC]). (D) Composite image of a cell with a normal signal pattern (2 signals for both the gold and red probes).](image)
of cells with chromosomal gains are morphologically abnormal. However, cells with relatively little or no morphologic atypia sometimes showed chromosomal abnormalities and/or HPV infection, and thus care was taken to be certain that these cells were also identified. Representative FISH images are shown in Figure 1.

**Determining Criteria for FISH Abnormality**

Receiver operator characteristics (ROC) curves that compared the sensitivity and specificity of the various cutoffs for the number of cells with gains of chromosomes (≥ 3 signals for 8q24 and/or 3q26) or the number of cells with HPV infection were used to determine the optimal cutoff(s) to consider a case as FISH positive. Combinations of cutoffs were also evaluated to determine whether the performance characteristics of the FISH assay could be improved over using 1 cutoff alone.

**Statistical Analyses**

Data were analyzed using the JMP 6.0 (SAS Institute Inc., Cary, North Carolina, U.S.A.) statistical software program. Student’s t test was used to compare the differences in the mean number of cells between histologic categories, and the McNemar test was used to compare differences in the sensitivity and specificity between the different assays. p Values < 0.05 were indicative of statistical significance.

**Results**

**FISH Quantitation**

Four different abnormalities identified by FISH were evaluated, including: total number of HPV infected cells; total number of cells with chromosomal gains of either 8q24, 3q26 or both; total number of cells showing both HPV infection and chromosomal gains; and total number of cells with integrated HPV infection. The number of cells with different types of FISH abnormalities in LSIL cytology specimens by histologic category is shown in Table I. This analysis shows that there is an increase in the mean number of cells with each type of FISH abnormality identified as the severity of histology diagnosis increased (negative vs. CIN 1 vs. CIN 2+).

<table>
<thead>
<tr>
<th>Variable</th>
<th>HPV(+) cells</th>
<th>Chrom(+) cells</th>
<th>Double(+) cells</th>
<th>Integrated HPV(+) cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biopsy diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>33</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>CIN 1</td>
<td>45</td>
<td>9</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>CIN 2+</td>
<td>96</td>
<td>21</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>p Value*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative vs. CIN 1</td>
<td>0.567</td>
<td>0.380</td>
<td>0.668</td>
<td>0.462</td>
</tr>
<tr>
<td>Negative vs. CIN 2</td>
<td>0.011*</td>
<td>0.005*</td>
<td>0.004*</td>
<td>0.003*</td>
</tr>
<tr>
<td>CIN 1 vs. CIN 2</td>
<td>0.032*</td>
<td>0.032*</td>
<td>0.008*</td>
<td>0.013*</td>
</tr>
</tbody>
</table>

*p < 0.05 = Statistically significant difference in the mean number of cells between the 2 categories by Student’s t test.

†Cells with chromosomal abnormalities (i.e., gains of 3q26 or 8q24).
‡HPV positive cells with chromosomal abnormalities.
biopsy results of negative, CIN 1 and CIN 2+, respectively. A similar trend was also identified with the mean number of cells with chromosomal gains (4, 9 and 21 for negative, CIN 1 and CIN 2+, respectively) and number of cells with concomitant HPV infection and chromosomal gains (2, 4 and 14 for negative, CIN 1 and CIN 2+, respectively) and number of cells with HPV integration (2, 4 and 13 for negative, CIN 1 and CIN 2+, respectively). Student’s t test demonstrated a significant difference in the mean number of cells with these abnormalities between biopsy diagnoses of negative vs. CIN 2+ and CIN 1 vs. CIN 2+ for all 4 FISH categories. There was no significant difference in the mean number of cells with these abnormalities between the biopsy diagnoses of negative and CIN 1 for all FISH categories.

**Cutoff Determination**

Separate ROC curves were produced using data from the number of cells with HPV infection and the number of cells with chromosomal gains (defined as 3 or more copies in at least 1 of the 2 probes) and are shown in Figure 2. An in-depth analysis of the data revealed that the number of HPV infected cells and the number of cells with chromosomal gains may provide complementary detection of CIN 2+ in these patients. The results from these 2 analyses were then used to guide a selection of combined FISH cutoffs that produced the highest sensitivity possible without sacrificing considerable specificity for the detection of CIN 2+ lesions from cytology specimens diagnosed as LSIL. After evaluating the performance characteristics of numerous cutoff combinations, the most favorable combination to consider a case positive for abnormality was when either ≥ 30 cells with HPV infection or ≥ 3 cells with chromosomal gains were identified. This combination of criteria was termed “FISH 1.” A second FISH cutoff of ≥ 3 cells demonstrating chromosomal gains (termed “FISH 2”) was also assessed to determine the performance characteristics of the assay using only the chromosomal portion of the FISH probe set.

**Comparison of HC2 and FISH for Detection of CIN 2+**

Patient demographics from specimens included in the comparison analysis are shown in Table II. Tables III and IV demonstrate the performance characteristics of HC2 and FISH for the detection of CIN 2+ in patients with an LSIL cervical cytology diagnosis. The performance characteristics of FISH were evaluated with FISH 1 and FISH 2 criteria. HC2 had a sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) of 100%, 28%, 100% and 32%, respectively, while the sensitivity, specificity, NPV and PPV of FISH 1 were 90%, 48%, 93% and 37%, respectively. A comparison of the 2 assays showed that the sensitivity of HC2 was not significantly different than that of FISH 1 (100% vs. 90%, p = 0.25), while the specificity of HC2 was significantly lower than that of FISH 1 (28% vs. 48%, p = 0.003). The NPV of HC2 was also higher than that of FISH 1 (100% vs. 93%, respectively), while the PPV was slightly lower (32% vs. 37% for HC2 and FISH 1, respectively).

A comparison of the second FISH cutoff of ≥ 3 cells with chromosomal gains (FISH 2) to FISH 1 and HC2 is also shown in Table III. FISH 2 had a sensitivity and specificity of 83% and 54%, respectively. The sensitivity of FISH 2 was not significantly different than that of HC2 (83% vs. 100%, p = 0.063) or FISH 1 (83% vs. 90%, p = 0.157). However, the specificity was significantly higher than that of both HC2 (54% vs. 28%, p < 0.001) and FISH 1 (54% vs. 48%, p = 0.025). FISH 2 diagnosed the lowest
The preliminary results of this study suggest that FISH testing may improve the ability to identify which patients with a cervical cytology diagnosis of LSIL are most likely to have or progress to CIN 2+ on clinical follow-up. Due to the higher specificity of FISH, the use of a positive FISH result as the criterion for sending a patient to colposcopy would have reduced the percentage of patients referred to colposcopy from 79% with HC2 testing to 62% with FISH (Table III). However, FISH had a slightly lower sensitivity and NPV for CIN 2+ lesions (90% and 93%, respectively) than HC2 (100% and 100%), and thus a small fraction of patients with a CIN 2+ lesion might be undiagnosed by FISH. Previous studies have shown that HPV testing is not effective for identifying which patients with a cervical cytology diagnosis of LSIL are likely to have a CIN 2+ lesion on colposcopic biopsy. The Atypical Squamous Cells of Undetermined Significance/Low-Grade Intraepithelial Lesion Triage Study (ALTS) concluded that HR-HPV testing is not practical for triaging patients with an LSIL diagnosis to colposcopy because 83% of these specimens are positive for HR-HPV, potentially resulting in an excess of patients referred to colposcopy. In addition, many LSIL lesions will spontaneously regress. Determining the appropriate clinical follow-up for these patients is problematic because 10–30% of LSIL patients will have or progress to a higher grade lesion, necessitating treatment. Current guidelines suggest that nearly all patients (excluding adolescents and postmenopausal women) with an LSIL cytology diagnosis should have a colposcopic examination. These recommendations are supported by data from the ALTS trial indicating that these women have a risk of developing CIN 2 or 3 similar to that of patients with an ASC-US diagnosis who have a positive HR-HPV result. The American Society of Colposcopy and Cervical Pathology conference concluded that these data support managing both groups with colposcopy. Colposcopy can be expensive and may sometimes be associated with physical and/or psychological harm to the patient. Thus, a test that can more accurately triage patients with an LSIL cytology diagnosis to colposcopy would be valuable. Since most of these individuals will not have a significant lesion on colposcopic examination, there is a need for a test that can accurately determine which patients are at highest risk of having CIN 2+ should undergo more aggressive treatment. Such a test could spare those low-risk patients from colposcopic examination and possible overtreatment. A reflex test could therefore reduce the overall cost of managing patients with an LSIL diagnosis and may provide the patient with an opportunity to have her lesion spontaneously regress.

The preliminary results of this feasibility study suggest that FISH may have the potential to identify which patients diagnosed with a cervical cytology result of LSIL will have or progress to CIN 2+ on clinical follow-up. When using the combined FISH cutoffs of ≥ 30 HPV+ cells or ≥ 3 cells with chromosomal gains identified (FISH 1), the sensitivity of detecting CIN 2+ was not significantly different than that of HC2 (90% vs. 100%, p = 0.25), while the specificity of FISH was significantly higher than

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity for CIN 2+ n (%)</th>
<th>Specificity for CIN 2+ n (%)</th>
<th>NPV n (%)</th>
<th>PPV n (%)</th>
<th>Predicted referral rate n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC2</td>
<td>29/29 (100)</td>
<td>24/86 (28)</td>
<td>24/24 (100)</td>
<td>29/91 (32)</td>
<td>91/115 (79)</td>
</tr>
<tr>
<td>FISH 1</td>
<td>26/29 (90)</td>
<td>41/86 (48)</td>
<td>41/44 (93)</td>
<td>26/71 (37)</td>
<td>71/115 (62)</td>
</tr>
<tr>
<td>FISH 2</td>
<td>24/29 (83)</td>
<td>46/86 (54)</td>
<td>46/51 (90)</td>
<td>24/64 (38)</td>
<td>64/115 (56)</td>
</tr>
</tbody>
</table>

FISH 1 = FISH analysis positive when ≥ 30 HPV+ cells or ≥ 3 cells with chromosomal gains identified.
FISH 2 = FISH analysis positive when ≥ 3 cells with chromosomal gains identified.
Predicted referral rate = proportion of specimens diagnosed as positive.

Table IV McNemar Test of Results in Table III

<table>
<thead>
<tr>
<th>Result</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC2 vs. FISH 1</td>
<td>0.25</td>
<td>0.003*</td>
</tr>
<tr>
<td>HC2 vs. FISH 2</td>
<td>0.063</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>FISH 1 vs. FISH 2</td>
<td>0.157</td>
<td>0.025*</td>
</tr>
</tbody>
</table>

*p<0.05 Statistically significant.
that of HC2 (48% vs. 28%, p = 0.003). FISH was able to maintain a high sensitivity (90%) and NPV (93%) for CIN 2+ while reducing the proportion of patients with a positive test result (predicted referral rate) in comparison to HC2 (62% vs. 79%).

HC2 testing had slightly higher sensitivity (though not significantly different) and higher NPV than FISH 1 for the detection of CIN 2+ (100% and 100% vs. 90% and 93% for HC2 and FISH 1). There were 3 patients with CIN 2+ who were positive by HC2 but negative by FISH 1. Two of these patients were found to have cervical biopsies with small fragments of CIN 2 in association with CIN 1. It is conceivable that these lesions were not adequately sampled by the cervical/endocervical brush due to their small size and therefore not detectable by the FISH assay. The third patient was found to have CIN 1 at the time of the original cervical cytology specimen and developed CIN 2 on clinical follow-up 9 months later. It is possible that the CIN 1 lesion had time to progress to CIN 2 during this time frame. Alternatively, any or all 3 of these patients may have had lesions that did not have genetic alterations that could be detected with the FISH probe set utilized in this study.

A detailed analysis of the FISH results was performed to determine if the HPV portion of the probe set is useful for the detection of high grade cervical lesions. The results in Table III highlight the test characteristics of using 2 different FISH cutoffs. The first cutoff (FISH 1: ≥ 30 cells with HPV infection or ≥ 3 cells with chromosomal gains) utilizes the results obtained from both the HPV and chromosomal aspects of the assay, while the second cutoff (FISH 2: ≥ 3 cells with chromosomal gains) utilizes only the results obtained with the chromosomal portion of the probe set. The sensitivity for the detection of CIN 2+ for FISH 1 was not significantly different than for FISH 2 (90% vs. 83%, p = 0.157), while the specificity of FISH 1 was significantly lower than that of FISH 2 (48% vs. 54%, p = 0.025). The HPV probes detected 2 additional patients with CIN 2+ when compared to using the chromosomal probes alone while producing a false positive diagnosis in 5 additional specimens without CIN 2+. Furthermore, the sensitivity of FISH 2 was not significantly different than that of HC2 (100% vs. 83%, p = 0.063), while the specificity of FISH 2 was nearly double that of HC2 (54% vs. 28%, p < 0.001). FISH 2 also had the lowest proportion of positive diagnoses (predicted referral rate) of all 3 test results (56%, 62% and 79% for FISH 2, FISH 1 and HC2, respectively). These preliminary data suggest that the HPV portion of this probe set may not add significant clinical value to this assay when used on this cohort of patients, especially since the HPV tyramide signal amplification associated with this assay is fairly laborious and expensive to perform.

While the HPV portion of the probe set may not add significant value to this assay for the detection of CIN 2+, it does have the ability to quantify other factors, such as HPV viral load and physical status (episomal vs. integrated), that may be clinically useful. HPV quantification with this FISH assay is different than other methods such as HPV quantification by PCR and HC2 because it can enumerate the number of HPV infected cells in a sample rather than measuring the number of HPV virions. Table I demonstrates that the mean number of cells with HPV infection enumerated in LSIL cytology specimens significantly increased in patients with CIN 2+ as compared to patients with less severe lesions (mean of 33, 45 and 96 for negative, CIN 1 and CIN 2+, respectively). The mean number of HPV infected cells was significantly higher in those with CIN 2+ when compared to patients with CIN 1 (p = 0.032) and negative (p = 0.011) biopsy results. Interestingly, the 3 patients found to have CIN 3 had > 100 HPV infected cells enumerated. This trend is also evident when looking at the mean number of cells with HPV integration (mean of 2, 4 and 13 for negative, CIN 1 and CIN 2+, respectively). The mean number of cells with HPV integration was significantly higher in those with CIN 2+ when compared to patients with CIN 1 (p = 0.013) and negative (p = 0.003) biopsy results. Further analyses of the data revealed that there were 16 patients with ≥ 10 cells with HPV integration and that 8 (50%) were found to have CIN 2+ on follow-up. Of the remaining 8 patients without CIN 2+, 5 were found to have a persistent low grade lesion during clinical follow-up. The other 3 patients had an initial biopsy diagnosis of CIN 1; of them, 2 patients were lost to follow-up, and 1 patient had a single negative cervical cytology specimen before being lost to follow-up. This suggests that quantifying HPV status and viral load may provide additional prognostic information in the follow-up of these patients. Further studies are needed to determine if HPV probes provide sufficient value to the test’s overall performance to justify the extra time and expense associated with maintaining the HPV probes in the probe set. It is possible that the performance char-
acteristics of the assay would be just as good or better if the HPV probes were removed and replaced with 1 or 2 additional chromosomal probes.

While the results of this study are encouraging, there are several limitations to consider. First, this study was performed in a retrospective manner only on patients with concurrent cytology and biopsy specimens, therefore potentially biasing the results due to the selection of a high-risk patient population. Second, the cutoff values used to consider a case positive by FISH were chosen to produce the optimal assay performance on this cohort of specimens, while HC2 analyses used preset cutoff values that were not adjusted to achieve the best possible test performance. This could have favorably biased the results obtained for the FISH assay. Third, the results of this study may have been impacted due to the manner in which the specimen was split for the 3 assays. As stated in the Materials and Methods, routine cytology was given access to the entire cervical cytology specimen for clinical testing. The remaining specimen was then split for HC2 and FISH testing. The ThinPrep method used to prepare the routine cytology slide may have removed the largest portion of the cells for some cases, leaving few cells for HC2 and FISH analysis. This could have compromised the results obtained with HC2 and FISH assays. Finally, the small sample size of the patient population selected may limit the statistical power of this study. A larger sample size may have resulted in statistically significant differences in the specificity and sensitivity between HC2 and FISH.

The results from this limited feasibility study suggest that FISH may have the ability to significantly decrease the number of individuals being referred to colposcopy due to an LSIL cytology diagnosis while maintaining sufficiently high sensitivity to ensure that most patients with a CIN 2+ lesion will undergo a colposcopic examination. This assay may potentially be most beneficial when used in young patients (<30 years) diagnosed with an LSIL cervical cytology result. These patients are most likely to harbor HR-HPV lesions (i.e., test positive for HR-HPV) but are at low risk of having a high grade cervical lesion. Therefore, many of these younger patients may undergo an unnecessary colposcopy procedure and would be most likely to benefit from having an efficient triage algorithm. Future prospective studies are needed to evaluate this assay on patients with low grade cervical lesions.

References


