

Clinical Correlation of APTIMA® HPV Assay in Comparison with Hybrid Capture 2® Test in Cervical Cancer Screening

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Background and Objective

HPV DNA tests have high clinical sensitivity for detection of cervical precancer and cancer, however they lack clinical specificity compared with Pap cytology. Malignant transformation is induced and maintained by HPV E6/E7 oncogenes. As a result, cervical dysplasia and carcinoma are associated with over expression of the oncogenes, and their activity can be detected through E6/E7 mRNA. Therefore, testing for E6/E7 mRNA could be more specific than testing for HPV DNA in identifying those at risk of cervical cancer. APTIMA HPV assay (Gen-Probe) is a recently developed test which detects E6/E7 mRNA of 14 oncogenic HPV types. This study was carried out to determine the clinical performance of APTIMA in comparison with Hybrid Capture 2 (HC2; Qiagen) which detects DNA of 13 oncogenic HPV types.

Methods

Women with cytologic abnormalities referred to colposcopy and those having routine Pap screening served as the study population. Cytology was performed with ThinPrep® method (Hologic) and cervical specimens were tested with APTIMA and HC2 tests. The study subjects underwent colposcopy, and if indicated, histologic diagnosis. APTIMA and HC2 results were correlated with cytologic and histologic grades. Histology confirmed high grade cervical intraepithelial neoplasia or worse (\geq CIN2) served as the gold standard.

Results

Of 1199 colposcopy referral cases studied, there were 363 cases diagnosed with \geq CIN 2. APTIMA showed a clinical sensitivity of 95% for detection of \geq CIN 2 vs. 93.9% for HC2 (Table 1). Discrepant analysis is shown in Tables 2a, 2b, and 2c.

Table 1. Clinical Sensitivity: APTIMA vs HC2
Colposcopy Referral Cases, N = 1199. \geq CIN2* = 363

| Test | | HC2 | | Total |
|--------|---|-----|----|-------|
| | | + | - | |
| APTIMA | + | 335 | 10 | 345 |
| | - | 6 | 12 | 18 |
| Total | | 341 | 22 | 363 |

APTIMA Sensitivity 345/363 = 95.0% (95% CI: 92.8, 97.3)
HC2 Sensitivity 341/363 = 93.9% (95% CI: 91.5, 96.4)
Agreement 347/363 = 95.6% (Kappa = 0.577)

* CIN 2 = Cervical Intraepithelial Neoplasia Grade 2

Table 2a. CIN2+ Cases Testing Negative by Both APTIMA & HC2 (N = 12)

| LBC | LA Genotype |
|------------|--------------------------------|
| 1. Normal | Negative |
| 2. Normal | Negative |
| 3. ASCUS* | Negative |
| 4. ASCUS | 70 |
| 5. LSIL** | 82 |
| 6. ASCUS | 61, 82 |
| 7. Normal | 16 |
| 8. LSIL | 16, 52, 70, 84 |
| 9. Normal | 56, 86 |
| 10. Normal | 42, 52, 53 |
| 11. Normal | 39, 62, 67, 89 |
| 12. Normal | 39, 42, 52, 53, 58, 61, 62, 73 |

*ASCUS = Atypical Squamous Cells of Undetermined Significance
**LSIL = Low Grade Squamous Intraepithelial Lesion

Table 2b. CIN2+ Cases Testing Negative by HC2 and Positive by APTIMA (N = 10)

| LBC | LA Genotype |
|------------|--------------------|
| 1. ASCUS | 16 |
| 2. ASCUS | 18, 52, 54 |
| 3. LSIL | 33, 62 |
| 4. Normal | 16, 51, 52 |
| 5. ASCUS | 16, 45 |
| 6. LSIL | 16, 89 |
| 7. HSIL* | 51, 53, 62, 81, 83 |
| 8. ASCUS | 51, 61 |
| 9. LSIL | 18 |
| 10. Normal | 18 |

*HSIL = High Grade Squamous Intraepithelial Lesion

Table 2c. CIN2+ Cases Testing Negative by APTIMA and Positive by HC2 (N = 6)

| LBC | LA Genotype |
|-------------|----------------|
| 1. ASCUS | 70 |
| 2. HSIL | 16, 18 |
| 3. ASCUS | 51, 61, 89 |
| 4. LSIL | 16, 25 |
| 5. ASCUS+H* | 52, 81, 83 |
| 6. ASCUS | 16, 31, 51, 82 |

*ASCUS = Atypical Squamous Cells of Undetermined Significance, Favour HSIL

The comparative specificity of APTIMA and HC2 is illustrated in Figure 1. Of 1120 routine screen population tested, 7 cases were diagnosed with \geq CIN 2, all of whom were detected by both APTIMA and HC2. Of the remaining 1113 with $<$ CIN1, APTIMA showed a higher clinical specificity than HC2 (Table 6).

Table 3. Clinical Specificity: APTIMA vs HC2
Colposcopy Referral Cases, N = 1199. \leq CIN 1*, N = 836 (69.7%)

| Tests | | HC2 | | Total |
|--------|---|-----|-----|-------|
| | | + | - | |
| APTIMA | + | 430 | 29 | 459 |
| | - | 74 | 303 | 377 |
| Total | | 504 | 332 | 836 |

APTIMA Specificity 377/836 = 45.1% (95% CI: 41.7, 48.5)
HC2 Specificity 332/836 = 39.7% (95% CI: 36.4, 43.0)
Agreement 733/836 = 87.7% (Kappa = 0.769)

* CIN 2 = Cervical Intraepithelial Neoplasia Grade 2

Table 4. Comparative Performance of APTIMA and HC2
Colposcopy Referral Cases, N=1199

| Tests | Sensitivity | Specificity | PPV |
|--------|---------------------|--------------------|--------------------|
| APTIMA | 95.0% (92.8, 97.3)* | 45.1% (41.7, 48.5) | 42.9% (39.5, 46.3) |
| HC2 | 93.9% (91.5, 96.4) | 39.7% (36.4, 43.0) | 40.4% (37.1, 43.7) |

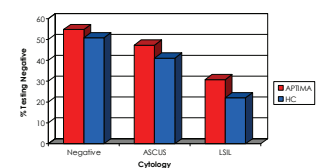
* 95% Confidence Interval

Table 5. Correlation of Cytology/Histology with APTIMA and HC2
Colposcopy Referral Cases, N = 1154

| LBC | Histology | N | APTIMA | | HC2 | |
|-------------------|--------------|-----|-------------|-------------|--------------|-------------|
| | | | + | - | + | - |
| Normal n = 363 | \geq CIN 2 | 58 | 50 (86.2%)* | 8 | 48 (82.8%)* | 10 |
| | \leq CIN 1 | 305 | 138 (45.2%) | 167 (54.8%) | 150 (49.2%) | 155 (50.8%) |
| ASCUS n = 340 | \geq CIN 2 | 82 | 77 (93.9%) | 5 | 76 (92.7%)* | 6 |
| | \leq CIN 1 | 258 | 138 (53.5%) | 122 (47.3%) | 152 (58.9%) | 106 (41.1%) |
| LSIL n = 308 | \geq CIN 2 | 100 | 97 (97%) | 3 | 95 (95%) | 5 |
| | \leq CIN 1 | 208 | 144 (69.2%) | 64 (30.8%) | 162 (77.4%) | 46 (22.1%) |
| HSIL n = 143 | \geq CIN 2 | 116 | 110 (94.8%) | 6 | 112 (96.3%)* | 4 |
| | \leq CIN 1 | 27 | 27 (100%) | 0 | 27 (100%) | 0 |

* Percentage

Figure 1. Specificity of APTIMA and HC2 \leq CIN 1 cases stratified by cytology
Colpo Referral Cases, N = 1154



Conclusions

- APTIMA HPV assay showed the same level of clinical sensitivity as HC2 for detection of \geq CIN 2 but with a higher clinical specificity.
- APTIMA HPV assay has the potential to reduce the proportion of women labeled as being HPV positive and unnecessarily subjected to intensified clinical management.
- The reduced specificity of HC2 could be attributable to cross-reactivity of HC2 with non-oncogenic types
- APTIMA HPV assay can serve as a reliable test both in cervical cancer screening and ASCUS triage.

Table 6. Clinical Specificity: APTIMA vs HC2 Routine Screen Population
N = 1120
 \geq CIN 2, = 7 (Disease prevalence, 0.6%); \leq CIN1 = 1113

| Tests | | Disease | |
|--------|---|---------|-----|
| | | + | - |
| APTIMA | + | 7 | 138 |
| HC2 | + | 7 | 174 |

Among those with no disease (mostly normal cytology), proportions testing positive:
APTIMA 138/1113 = 12.4%
HC2 174/1113 = 15.6%*

* Statistically significant

APTIMA showed a clinical specificity of 45.1% vs. 39.7% for HC2 (Table 3). The comparative performance of APTIMA and HC2 is summarized in Table 4. The correlation of cytology/histology with APTIMA and HC2 is shown in Table 5.