

# 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  $>$  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)

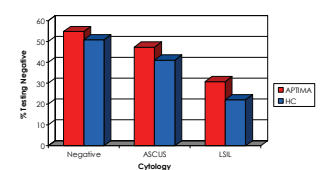
\* 95% Confidence Interval

**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 (77.9%)	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.