APTIMA® HPV ASSAY PERFORMANCE: Correlation Between Detection of Human Papillomavirus E6/E7 mRNA and Clinically Relevant Cervical Disease Detection

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Funding for graphics and content dissemination provided by

Every life is extraordinary

Dr. Einstein has advised or participated in educational speaking activities, but does not receive an honorarium from any companies, including for the writing of this supplement. Montefiore has received grant funding for research-related costs of clinical trials that Dr. Einstein has been the overall or Montefiore PI from Merck, GSK, Roche, BD Diagnostics, and Hologic.
Funding for graphics and content dissemination provided by Hologic, Inc.

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RELATIONSHIP BETWEEN HPV INFECTION AND CERVICAL CANCER

The discovery of the role of human papillomavirus (HPV) as a necessary etiologic agent in the development of cervical cancer 35 years ago marks a key discovery in our understanding of cervical cancer, and the eventual development of screening and prevention strategies to target HPV. In particular, it has resulted in important leaps in the development of a new primary prevention strategy of HPV vaccination and utilizing molecular methods to assist providers in screening and management of precancerous lesions. Although there are more than 100 types of HPV, 14 types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) have oncogenic potential and are considered “high-risk” HPV types (HR-HPV); of these HR-HPV types, HPV 16 and 18 are the types responsible for 70% of cervical cancers worldwide.1

HPV is the most common sexually transmitted infection, and about 80% of sexually active women are estimated to become infected with one or more HPV types in their lifetime.2 Young women are more likely to be infected with HPV, but when compared to older women, are also more likely to have regression of clinically detectable infection.3,4

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MOLECULAR BIOLOGY OF HPV INFECTION

HPV infection of the cervix most often originates from skin to skin contact, typically from exposure through sexual activity. Once in the female reproductive tract, the virus infects cervical squamous...
epithelial cells. In infected cells, HPV DNA (~8 kb) can remain either in episomal form (free) or integrate into the cell genome.\textsuperscript{5,6} Integration often induces the expression of HPV E6 and E7 mRNA and proteins (Fig. 1). These oncoproteins interact with various proteins of the host cell (which they can inhibit or degrade), leading to the inability of the cell to inhibit apoptosis and allow for DNA repair.\textsuperscript{7} For instance, E6 protein causes degradation of the tumor suppressor protein p53, increasing the rate of random mutations that can lead to transformation.\textsuperscript{8} E7 inhibits the function of the Rb protein which regulates the cell cycle,\textsuperscript{9} which, when inhibited, can also drive the cell into a path of neoplastic transformation (Fig. 1).

Thus, while the presence of HPV DNA indicates the existence of an infection, the presence of E6/E7 mRNA indicates that the infection has become more biologically and likely clinically “active,” i.e., potentially oncogenic. The key involvement of E6 and E7 in oncogenesis is supported by the fact that E6 and E7 mRNAs are expressed in cervical cancer cells\textsuperscript{10} and the expression of E6 and E7 mRNAs increases with increasing severity of cervical disease.\textsuperscript{11} Moreover, many studies have shown the association between E6/E7 expression and cell transformation and the maintenance of a transformed phenotype.\textsuperscript{7} Altogether, these findings suggest that E6/E7 mRNAs are excellent markers

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**Figure 1. Molecular biology of HPV infection.** HPV viral particles enter cervical epithelial cells. In active infections, the viral genome integrates into the host cell genome. This leads to the expression of HPV E6 and E7 mRNA and oncoproteins, which induces carcinogenesis.
DISEASE PROGRESSION

The development of cervical cancer from the initial HPV infection is illustrated in Figure 2. Initial HPV infections can often manifest themselves as an abnormal cervical cytology, such as atypical squamous cells of undetermined significance (ASC-US) or low-grade squamous intraepithelial lesion (LSIL) cytology, shortly after infection of cervical epithelial cells. In most cases, these low-grade lesions are merely cytomorphological manifestations of a transient HPV infection, and most often regress. Rarely, often over many years, do they progress to a high-grade cervical intraepithelial neoplasia (CIN). Progression from a high-grade lesion to cervical cancer takes over a decade. It appears to take longer for a CIN3 lesion to evolve into cervical cancer than for an initial infection to evolve into a high-grade lesion.1

Persistence of HPV infection is mandatory for the transformation of low-grade lesions into high-grade lesions. As most (70%) HPV infections regress within 1 year after detection of the virus,12-14 so do most equivocal CIN lesions: of the CIN2 lesions, 40% are likely to regress within 2 years.15 Once CIN3 (or worse) lesions have developed, regression is unlikely and this needs to be treated.16

APTIMA HPV ASSAY

The Aptima HPV assay (Hologic, Inc.) was developed to qualitatively detect E6/E7 mRNA from 14 HR-HPV types (HPV
As shown in multiple prospective cohorts, HPV testing is typically more sensitive than cytology in a screening population. A study of compiled data from European and North American studies comparing cytology and HPV testing showed that cytology alone has a much lower sensitivity than screening strategies that include both cytology and HPV testing.1

**Speciﬁcity**

The Aptima HPV assay has shown an excellent, if not greater speciﬁcity, than other HPV DNA-based tests. This suggests that Aptima HPV might yield fewer false positive results than HC2 in a screening population. In a pooled analysis of eight studies, the Aptima HPV assay had a signiﬁcantly higher speciﬁcity than the HC2 reference test for the detection of CIN2+ in women with ASC-US (Table 1).18 Even in an LSIL population, it appears that in older women, HPV testing might offer improved triage risk stratiﬁcation. Because of its improved speciﬁcity, the Aptima HPV test might also offer such risk stratiﬁcation. This higher speciﬁcity of the Aptima HPV test has the potential to help reduce the number of unnecessary colposcopies, which are a recognized harm to patients in the most recent treatment guidelines.16

**Aptima HPV assay is in compliance with guidelines**

The Aptima HPV assay has been approved by the U.S. Food and Drug Administration (FDA) for the detection of HPV in clinical samples (ThinPrep® Liquid Cytology Specimens). For the assay to be used internationally, it is required to meet international guidelines mandating that the assay meets the criterion of non-inferiority in comparison to a reference assay as well as intra- and inter-laboratory reproducibility criteria.32 A recently published study showed that the Aptima HPV assay had a clinical sensitivity and speciﬁcity similar to that of a GP5+/6+-PCR-based assay (P = 0.03 and P = 0.00016, respectively), an intra-laboratory reproducibility over time of 6.0% (kappa 0.8) and an inter-laboratory agreement of 6.7% (kappa 0.1).33 Thus, the Aptima HPV assay meets the cross-sectional clinical and reproducibility criteria of the international guidelines for HPV test requirements for cervical screening.

**This clinically validated mRNA assay uses a different technology as compared to other clinically validated HPV DNA tests.**

Figure 3. Sensitivity of Aptima HPV versus DNA-based HPV tests – Data from the literature. HC2 = Digene® Hybrid Capture® 2 HPV DNA Test®; Cobas = Roche Cobas® AMPLICOR® HPV Test.17, 20-30

16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) based on the fact that the presence of HPV E6/E7 mRNAs is an excellent indicator of cervical disease. This clinically validated mRNA assay uses a different technology as compared to other clinically validated HPV DNA tests. The assay involves three main steps, which take place in a single tube at one temperature: (i) target capture of the target mRNA using capture oligomers and magnetic microparticles; (ii) target mRNA amplification using Transcription-Mediated Amplification; and (iii) detection of the amplification products (amplicons) using the Hybridization Protection Assay.17

**Sensitivity**

The sensitivity of the Aptima HPV assay for the detection of CIN3 or worse lesions has been reported to be higher than 90%, and statistically equivalent to that of DNA-based tests in comparative studies performed in referral and screening populations (Fig. 3). A meta-analysis showed that the sensitivity of the Aptima HPV test is equivalent to that of Hybrid Capture 2, the reference HPV test, when the two assays were compared side-by-side for the detection of CIN2+ or CIN3+ lesions in women with ASC-US or LSIL cytology (Table 1).18

Table 1. Pooled relative sensitivity and speciﬁcity of Aptima HPV vs. HC2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASC-US CIN2+</td>
<td>1.01 (0.7-1.06)</td>
<td>1.1 (1.08-1.31)</td>
</tr>
<tr>
<td>ASC-US CIN3+</td>
<td>1.01 (0.6-1.06)</td>
<td>1.18 (1.08-1.2)</td>
</tr>
<tr>
<td>LSIL CIN2+</td>
<td>0.6 (0.2-1.03)</td>
<td>1.37 (1.22-1.54)</td>
</tr>
<tr>
<td>LSIL CIN3+</td>
<td>0.8 (0.1-1.06)</td>
<td>1.35 (1.11-1.66)</td>
</tr>
</tbody>
</table>

Reproduced (without modification) from Arbyn 2013 article (reference 18) with the authors’ permission. Speciﬁcity data are pooled data from references 17, 20, 23-25, 27, 28, 31.
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A recently published study showed that the Aptima HPV assay had a clinical sensitivity and specificity similar to that of a GP5+/6+-PCR-based assay (P = 0.039 and P = 0.00016, respectively), an intra-laboratory reproducibility over time of 96.0% (kappa 0.89) and an inter-laboratory agreement of 96.7% (kappa 0.91).\textsuperscript{33} Thus, the Aptima HPV assay meets the cross-sectional clinical and reproducibility criteria of the international guidelines for HPV test requirements for cervical screening.

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**Table 1.** Pooled relative sensitivity and specificity of Aptima HPV vs. HC2

<table>
<thead>
<tr>
<th>Triage group</th>
<th>Outcome</th>
<th>Parameter</th>
<th>Ratio (APTIMA/HC2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASC-US</td>
<td>CIN2+</td>
<td>Sensitivity</td>
<td>1.01 (0.97-1.06)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Specificity</td>
<td>1.19 (1.08-1.31)</td>
</tr>
<tr>
<td>CIN3+</td>
<td>Sensitivity</td>
<td>1.01 (0.96-1.06)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Specificity</td>
<td>1.18 (1.08-1.29)</td>
<td></td>
</tr>
<tr>
<td>LSIL</td>
<td>CIN2+</td>
<td>Sensitivity</td>
<td>0.96 (0.92-1.03)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Specificity</td>
<td>1.37 (1.22-1.54)</td>
</tr>
<tr>
<td>CIN3+</td>
<td>Sensitivity</td>
<td>0.98 (0.91-1.06)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Specificity</td>
<td>1.35 (1.11-1.66)</td>
<td></td>
</tr>
</tbody>
</table>

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It appears that in older women, HPV testing might offer improved triage risk stratification.
Recent guidelines for the prevention and detection of cervical cancer by the American Cancer Society, the American Society for Colposcopy and Cervical Pathology, and the American Society for Clinical Pathology in consensus with a number of other scientific and patient stakeholders\(^3\(^4\) as well as the abnormal screening test management guidelines\(^6\) recommend a preferred strategy of using HPV testing in conjunction with cytology (a strategy termed ‘co-testing’) for primary screening in women 30 to 65 years. Incorporating HPV testing in primary screening strategies is based on the higher sensitivity and negative predictive value of HPV testing when compared to cytology. The benefit of incorporating HPV testing into primary screening would be to increase disease detection while reducing the number of unnecessary colposcopies, which is a recognized harm to patients. The criteria set forth in the guidelines for HPV testing are that (i) the HPV test must detect HR (oncogenic) HPV types, mainly HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59; (ii) the HPV test must have been analytically and clinically validated with proven acceptable reproducibility, clinical sensitivity, specificity, and positive and negative predictive values for CIN2+, as documented by U.S. FDA licensing and approval or publication in peer-reviewed scientific literature;\(^1\(^6\) (iii) sensitivity of HPV testing for CIN3+ and CIN2+ should be greater than or equal to 90%, and (iv) the percentage of women in the general population who test (screen) positive, as a measure of false positive results, should be less than or equal to established thresholds from well-validated HPV DNA tests.\(^3\(^4\) The Aptima HPV assay meets all of these criteria and is suitable for use in screening and co-testing strategies for the detection of cervical cancer, according to the most recent U.S. guidelines. When used as per guidelines, screening strategies utilizing Aptima HPV and other highly sensitive molecular HPV tests ultimately improve the identification of clinically worrisome cervical disease and help risk-stratify those women who might need shorter interval follow-up. This helps focus on those few women at significant risk of cervical cancer, while safely informing providers of the many who can be managed conservatively, which ultimately minimizes patient harm.

The benefit of incorporating HPV testing into primary screening (co-testing) would be to increase disease detection while reducing the number of unnecessary colposcopies...

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**The Aptima HPV assay is suitable for use in screening and co-testing strategies for the detection of cervical cancer, according to the most recent U.S. guidelines.**

Published as a promotional supplement to *Contemporary OB/GYN*
ACKNOWLEDGEMENTS

The author would like to acknowledge Florence Paillard for her editorial assistance.

REFERENCES


