

Testing of Integrated Human Papillomavirus mRNA Decreases Colposcopy Referrals: Could a Change in Human Papillomavirus Detection Methodology Lead to More Cost-Effective Patient Care?

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Key Words

Cervix · Human papillomavirus · Cervical cancer screening · Integrated mRNA · APTIMA · Hybrid capture · ASCUS triage · Squamous neoplasia · Virology · Molecular and diagnostic gynecologic cytopathology

Abstract

Objectives: This study investigates potential colposcopy referral rates, as per the latest American Society for Colposcopy and Cervical Pathology recommendations, following the change in high-risk human papillomavirus (HR-HPV) detection methodology from Hybrid Capture 2 (HC2) to APTIMA at our institution. **Study Design:** Rates of colposcopy referral were compared between two cohorts, each comprising all Pap samples with a diagnosis of atypical squamous cells of undetermined significance (ASCUS) tested for HR-HPV in our laboratory during a 12-month period. Cohorts I and II included Pap samples tested with HC2 (n = 1,856) and APTIMA (n = 1,651), respectively. The rates of quantity not sufficient (QNS) results were determined for all Pap samples during the same time periods. **Results:** The proportion of HR-HPV-positive Pap samples with an ASCUS diagnosis was significantly

lower with APTIMA (42%) than with HC2 (53%; p < 0.0001). APTIMA also resulted in a significantly lower QNS rate among all Pap samples (0.42 vs. 4.3% with HC2; p < 0.0001). **Conclusion:** The change in HR-HPV detection methodology from HC2 to APTIMA has led to a 21% reduction in colposcopy referrals and a 90% decrease in QNS rates at our institution. The new methodology has resulted in more cost-effective patient care and fewer insufficient samples requiring repeat HR-HPV testing.

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Introduction

In April 2012, after the USA-FDA approval in 2011 [1], Fletcher Allen Health Care, in affiliation with the University of Vermont, adopted the APTIMA (Gen-Probe Inc., San Diego, Calif., USA) high-risk human papillomavirus (HR-HPV) messenger RNA (mRNA) assay.

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APTIMA has been widely used in Europe since 2008 [2]. Previously, Hybrid Capture 2 (HC2; Qiagen, Gaithersburg, Md., USA) was used for HR-HPV testing at our institution.

Through detection of HR-HPV DNA, HC2 detects transient as well as integrated viral infections. Transient HPV infections are common but self-limited and of less clinical importance. The presence of HR-HPV DNA does not distinguish the infections at highest risk of progressing to squamous dysplasia and neoplasia [3, 4].

Rather than detecting viral DNA, the APTIMA assay detects overexpression of HR-HPV viral E6 and E7 mRNA [2]. Through the detection of viral mRNA, the assay specifically targets HPV infections with viral DNA integrated into the host genome and, therefore, at highest risk of progressing to squamous carcinoma as HR-HPV integration is an important event in the carcinogenesis of cervical squamous carcinoma [3–5].

The APTIMA HR-HPV assay utilizes transcription-mediated amplification for the qualitative detection of E6 and E7 viral mRNA from 14 HR-HPV subtypes [2]. Although the assay does not discriminate between the subtypes, Gen-Probe offers a separate FDA-approved assay that distinguishes HR-HPV subtypes 16 and 18/45. An overexpression of E6 and E7 viral oncogenes leads to cell-cycle alterations through the expression of proteins that alter p53 and retinoblastoma proteins [6, 7]. HR-HPV integration into the host genome is an important event in the carcinogenesis of cervical squamous carcinoma [3–4, 7]. Through the detection of viral mRNA, the APTIMA assay targets persistent HPV infections that have viral DNA integrated into the host genome.

The APTIMA assay is performed in a single tube utilizing three main steps: target capture, target amplification and detection of amplification products [2]. Target capture isolates viral mRNA from a specimen using oligomers containing complementary sequences of HR-HPV mRNA regions linked to magnetic microparticles [2]. Transcription-mediated amplification technology amplifies HPV mRNA utilizing reverse transcriptase to generate a DNA copy of the target mRNA, and then T7 RNA polymerase to generate multiple copies of the RNA amplicon from the DNA copy template [2]. In the final step, the amplicon products are detected via chemiluminescent labels [2].

The French APTIMA Screening Evaluation (FASE) [8] is the first large-scale study in which APTIMA was compared with HC2 in a screening setting. The study involved a cohort of 5,006 women, aged 20–65 years, screened in Paris, France. A cervical sample from each

patient was tested with liquid-based cytology as well as both APTIMA and HC2 HR-HPV assays. The findings were correlated with surgical biopsies in patients with abnormal results. APTIMA and HC2 were both more sensitive for cervical intraepithelial neoplasia grade 2 (CIN2; 92.0 and 96.7%) and CIN3 (95.7 and 95.3%) than liquid-based cytology (69.1 and 73.3%, respectively) [8]. The specificity of APTIMA was significantly higher than that of HC2 (by 5.4–8.3%) in all categories [8].

Several other studies have indicated that APTIMA has increased specificity but similar sensitivity to HC2 for the detection of high-grade lesions [9, 10]. In a recent meta-analysis including eight studies comprising 1,839 cases of atypical squamous cells of undetermined significance (ASCUS) and 1,887 cases of low-grade squamous intraepithelial lesion (LSIL), Arbyn et al. [10] calculated the pooled sensitivities and specificities of both APTIMA and HC2 for the detection of CIN2 or worse. In cases of both ASCUS and LSIL, they found APTIMA to be equally sensitive as HC2, but more specific for detecting CIN2 or worse. On average, APTIMA was 19% more specific in the triage of ASCUS and 37% more specific in the triage of LSIL, and these findings were statistically significant [10]. Their findings indicate that the APTIMA test captures as many high-grade lesions as HC2, but with fewer false positive results.

In this study, the performance of APTIMA in HR-HPV testing following an ASCUS Pap diagnosis was compared with HC2. Both methodologies were also evaluated for quantity not sufficient (QNS) results in the testing of all Pap samples. Consideration was given to any impact on healthcare costs resulting from the change in the HR-HPV detection methodology used in the laboratory.

Materials and Methods

Case Selection

Data were retrieved from the pathology laboratory information system at Fletcher Allen Health Care. Case selection for the analysis of colposcopy referral included all patients undergoing HR-HPV testing with a concurrent ASCUS Pap diagnosis. Cohort I consisted of all Pap samples with an ASCUS diagnosis tested with the HC2 methodology (n = 1,856) between April 1, 2011 and March 31, 2012. Cohort II included those with an ASCUS diagnosis tested with the APTIMA methodology (n = 1,651) during the subsequent 12 months.

Case selection for the analysis of QNS rates included all Pap samples, not only those associated with an ASCUS diagnosis. Therefore, all Pap samples tested for HR-HPV with HC2 (n = 17,318) between April 1, 2011 and March 31, 2012 and all Pap samples tested with APTIMA (n = 16,554) between April 1, 2012 and March 31, 2013 were included.

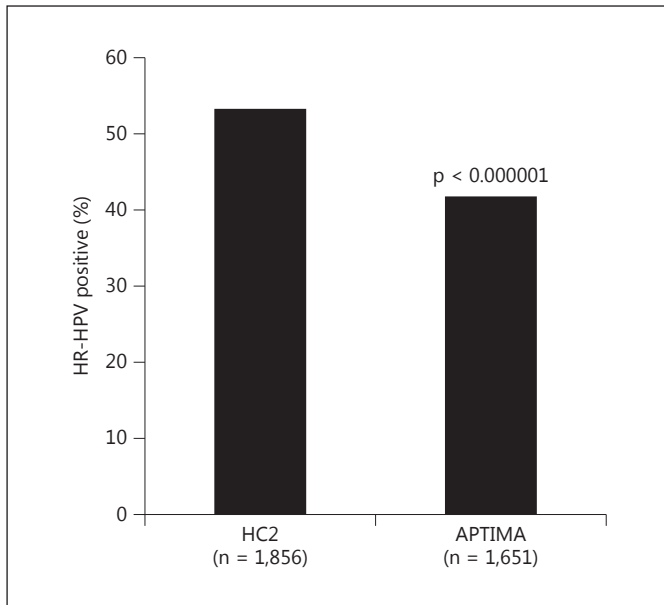


Fig. 1. HR-HPV positivity in ASCUS Pap samples tested with HC2 and APTIMA.

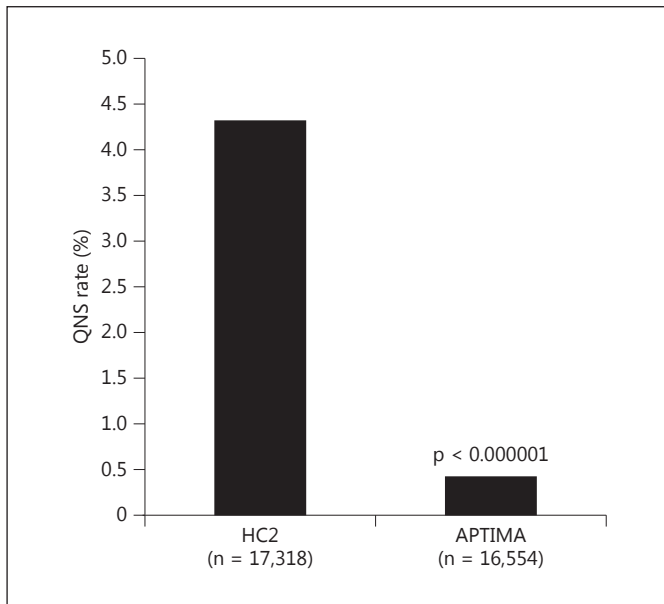


Fig. 2. Proportion of QNS results in all Pap samples tested with HC2 and APTIMA.

Patient Population

This center performs the majority of the cytopathology and HR-HPV testing for a large geographic region, as there are few competing laboratories. The patient demographics, number of providers and cytopathology staff remained constant over the time period of this study.

Data Analysis

The change in the colposcopy referral rate was calculated by determining the percentage of patients with an ASCUS Pap diagnosis and a positive HR-HPV test using the APTIMA methodology versus HC2. The change in the QNS rate was calculated by determining the percentage of QNS results of all Pap samples (not only those with ASCUS diagnoses) during the time periods of each cohort. Since the numbers of samples in each cohort are not identical, this study evaluated differences in proportions and changes in the rate rather than absolute numbers.

Statistical Analysis

The 95% confidence limits and the significance of the difference in the proportion of positives for the two cohorts were calculated for both the HR-HPV-positive rate in Pap samples with ASCUS diagnoses and the HR-HPV test QNS result rate for all Pap samples [11]. A χ^2 test was performed (no statistical software was used). A p value <0.05 was considered to be statistically significant.

Results

Of the Pap samples with an ASCUS diagnosis tested with HC2, 53% (n = 1,856) were positive for HR-HPV, whereas 42% (n = 1,651) were positive with the APTIMA assay (fig. 1). The difference in the proportion of positive HPV tests in the two cohorts was 11% (95% confidence limits 8–15%). This difference is statistically significant ($p < 0.0001$). These findings represent a 21% reduction in the potential colposcopy referral rate in the 12 months following the change to the APTIMA test.

During the time period of cohort I, 4.3% of all Pap samples (n = 17,318) tested for HR-HPV using HC2 were QNS. Only 0.42% of all Pap samples (n = 16,554) tested with APTIMA during the time period of cohort II were QNS (fig. 2). The difference in the proportion of QNS samples in the two cohorts is 3.9% (95% confidence limits 3.6–4.2%). This difference is statistically significant ($p < 0.0001$). These findings represent a 90% reduction in the QNS rate by switching technology.

Discussion

After our laboratory switched to the APTIMA methodology, there was a decrease in HR-HPV positivity of Pap samples with an ASCUS diagnosis, and a potential 21% reduction in the colposcopy referral rate in this patient population. Since HR-HPV testing is utilized for the triage of patients with ASCUS Pap diagnoses to colposcopic evaluation, a similar reduction in costs associ-

ated with colposcopy is anticipated. These costs include charges for clinic visits and the procedure itself as well as cytopathology, surgical pathology and other laboratory charges.

All of the HR-HPV testing in our laboratory is done in microbiology rather than in cytopathology. Even though the change to the APTIMA methodology required some training, the technologists currently performing the assay in our laboratory are not specifically trained in molecular techniques. Formal time-motion studies were not performed when the laboratory adopted APTIMA; however, there were notable improvements in the microbiology laboratory such as higher throughput, decreased hands-on time as well as the lower QNS rate with the change in HR-HPV detection methodology.

HC2 testing was performed on the Qiagen Rapid Capture System (RCS), which has a capacity of 384 specimens in one 4-hour run, and is fairly time consuming and tedious to set up. Depending on the volume, the HC2 test on the RCS requires 90–120 min of technologist hands-on time prior to the run and, as the luminometer for result detection is not interfaced with the RCS instrument, approximately 45–80 min for the technologist to manually record the results for each specimen.

Previously, with HC2, one technologist was devoted to HR-HPV testing. With the change to APTIMA, the technologist performing *Chlamydia trachomatis* and *Neisseria gonorrhoeae* testing absorbed the HR-HPV testing, since both assays are performed on the Tigris instrument (Hologic; Gen-Probe Inc., San Diego, Calif., USA). The Tigris machine has the capacity to run far more samples each day than RCS. However, to run the Tigris to full capacity (up to 1,000 tests per day including controls), it would require more than one 8-hour shift to report all the results. The technologist hands-on time to load specimens and reagents for the APTIMA/Tigris system is approximately 45 min, and then the instrument completes the run in approximately 4 h. The target amplification step requires slightly more technologist time to perform the cleaning involved in the transfer process compared to HC2, which was only a signal amplification assay. However, the HC2 assay required much more hands-on time throughout the run.

Training the technologist staff to run the APTIMA assay was relatively simple since the microbiology laboratory already had well-trained personnel who had been running the Tigris instrument for several years. Adding a very similar assay did not take much effort as the APTIMA assay is easy to learn, and the instrument provides helpful feedback if reagents are not loaded

properly. A new technologist would require approximately 5–10 days to be trained on the APTIMA assay in our laboratory.

In this study, the potential colposcopy referral rate is based on the American Society for Colposcopy and Cervical Pathology (ASCCP) recommendation that all HR-HPV-positive ASCUS Pap results should be referred to colposcopy. Although this center performs both Pap test interpretation and HR-HPV testing for a wide catchment area, including the entire state of Vermont as well as a large portion of upstate New York, the surgical pathology interpretation of cervical biopsies is performed at multiple local laboratories. Therefore, HR-HPV positivity in ASCUS Pap samples was used as a surrogate marker for likely Pap test referrals to colposcopy.

This study identified an additional area of cost savings in the 90% reduction rate of QNS results with the APTIMA methodology. This is not surprising, since the APTIMA test requires less sample volume than what is required for HC2 (1 vs. 5 ml). Since fewer patients will require repeat HR-HPV testing with the APTIMA methodology, there is an anticipated additional cost saving to the healthcare system. Furthermore, with the APTIMA methodology, there is a 23% reduction in reagent costs across all patients who receive HR-HPV tests, resulting in a direct cost saving to the laboratory.

There was an overall decrease in the number of ASCUS Pap diagnoses in the APTIMA cohort ($n = 1,651$) compared to the HC2 cohort ($n = 1,856$), and an overall decrease in HR-HPV testing during the time period of the two cohorts ($n = 16,554$ and $17,318$, respectively). The reduction in both overall Pap samples and Pap samples with an ASCUS diagnosis in this study reflects the current trend of fewer Pap tests being performed annually. This decline is an expected consequence of adherence by clinicians to the recent ASCCP guidelines, both those released in 2006 and in 2012. Since our laboratory performs the majority of Pap test interpretation and HR-HPV testing in the region, the decreased number of ASCUS Pap diagnoses over the time of this study is not likely due to a change in patient population.

The findings of this study, specifically a 21% reduction in the colposcopy referral rate, are consistent with the meta-analysis conducted by Arbyn et al. [10], which found an approximately 19% difference in the specificity between the two tests, with APTIMA having a greater specificity despite a similar sensitivity of >95%. We were unable to determine the sensitivities and specificities for the two HR-HPV tests as all surgical pathology for the area is not received at our laboratory.

The change in the HR-HPV detection methodology from HC2 to APTIMA has resulted in a decrease in the percentage of ASCUS and QNS diagnoses in this population. Consequently, there has been a reduction in the number of women requiring a referral for colposcopic examination as well as a reduction in the need for repeat HR-HPV testing, resulting in more cost-effective patient care.

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Disclosure Statement

The authors declare no conflicts of interest.

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