The Efficacy of the Aptima HPVmRNA Assay in Comparison to the Hybrid Capture II: a systematic review

Amanda A. Roy

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The Efficacy of the Aptima HPV mRNA Assay in Comparison to the Hybrid Capture II: a systematic review

Abstract

Background: Cervical cancer remains a leading cause of morbidity and mortality for women worldwide. Cytological methods of screening introduced over 50 years ago remain the primary screening method. With advancing science and the understanding of the etiology of cervical cancer, new methods of screening have been introduced such as HPV DNA and HPV mRNA testing. This review of literature focuses on the comparison between the APTIMA mRNA test and the Hybrid Capture II DNA test, two promising tests approved by the FDA.

Method: An exhaustive literature search was conducted in Medline, CINAHL, Web of Science, Google Scholar, and EBMRmultifile using the search terms papillomaviridae, uterine cervical dysplasia, RNA, and sensitivity and specificity in combination and alone as well as terms known to be synonymous. No limitations were placed on the search. Excluded were articles that assessed HPV screening in the HIV population, non-cervical methods of screening, screening conducted on self-collected samples, and those studies not utilizing both the APTIMA HPV mRNA assay and the Hybrid Capture II test, as well as articles not written in the English language.

Results: Seven accuracy studies were included based on the inclusion and exclusion criteria delineated in the methods section. All studies utilized histology as the gold standard of comparison. All studies included showed the Aptima HPV mRNA assay (AHPV) to have comparable sensitivity to Hybrid Capture II DNA test (HC2) and statistically better specificity for detection of clinically significant cervical lesions classified as CIN2+. Four of the studies compared the AHPV to both HC2 and liquid based cytology (LBC) and demonstrated AHPV and HC2 to have better sensitivity for CIN2+ than LBC.

Conclusion: This systematic review clearly shows that the AHPV assay could play a promising role in the future of cervical cancer detection. The AHPV maintained high sensitivity, similar to the HC2, while showing improved specificity.

Keywords: papillomaviridae, papillomavirus, uterine cervical dysplasia, cervical intraepithelial neoplasm, uterine cervical neoplasm, RNA, and sensitivity and specificity

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Master of Science in Physician Assistant Studies

First Advisor
Annjanette Sommers, PA-C, MS

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HPV mRNA, Aptima, Hybrid Capture II

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The Efficacy of the Aptima HPVmRNA Assay in Comparison to the Hybrid Capture II: a systematic review

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A Clinical Graduate Project Submitted to the Faculty of the
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Clinical Graduate Project Coordinator: Annjanette Sommers, PA-C, MS
Biography

[Information redacted for privacy]
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To my parents, *Stephen and Jill Roy*, Thank you for raising me to be compassionate to all creatures, stubborn and strong-willed, to never say never, and mostly importantly, to never give up on the things which are important to you. I would never be where I am now without you.

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Figure 2: Research Methodology
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List of Abbreviations

ACOG……………………………..American College of Obstetricians and Gynecologists
AHPV……………………………………………………APTIMA HPV
ASCUS………………………………Atypical Squamous Cells of Undetermined Significance
CI…………………………………………………………Confidence Interval
CIN……………………………………………………Cervical Intraepithelial Neoplasm
DNA……………………………………………………Deoxyribonucleic Acid
DTS………………………………………………….Direct Tube System
FDA……………………………………………………Food and Drug Administration
HPV……………………………………………………Human Papillomavirus
HC2……………………………………………………Hybrid Capture II
LBC………………………………………………….Liquid Based Cytology
LEEP……………………………………………Loop Electrosurgical Excision Procedure
LR+………………………………………………Positive Likelihood Ratio
LR-………………………………………………..Negative Likelihood Ratio
mRNA……………………………………………..Messenger ribonucleic acid
WHO……………………………………………….World Health Organization
The Efficacy of the Aptima HPVmRNA Assay in Comparison to the Hybrid Capture II: a systematic review

BACKGROUND

Cervical cancer remains a leading cause of morbidity and mortality worldwide with the World Health Organization (WHO) reporting approximately half a million women diagnosed with cervical cancer and over a quarter million deaths attributed to this preventable disease each year. Large disparities between developing/developed nations as well as rural/urban demographics are noted. The largest incidence occurs in developing countries where screening programs are not currently in place due to a lack of infrastructure and trained personnel inhibiting initiation of traditional cytology based screening (see Figure 1). This elucidates the further need for both refinement of current screening mechanisms and feasible options for areas without such systems in place.

Despite all of the advances made in industrialized nations, the American Cancer Society reported that in 2011 approximately 12 000 US women were diagnosed with cervical cancer leading to over 4000 deaths. Over $570 million of our annual healthcare burden is attributed to the diagnosis and treatment of pre-malignant lesions such as the estimated 412 000 CIN1 and CIN2/3 lesions and upwards of 3 million ASCUS cytologies which require further diagnostic workup.

The American College of Obstetricians and Gynecologists (ACOG) and the US Preventive Services Task Force currently recommend screening intervals of cytology every 2-3 years and HPV co-testing after age 30. Cytology based methods of screening were introduced over 50 years ago and have changed little over time. There are several well established weaknesses with the standard cytology based methods such as results are dependent on adequate collection of the specimen, interpretation is subjective in nature
and labor intensive, and the fact that a low negative predictive value requires frequent screening. In addition, liquid based cytology testing has been shown to have a sensitivity of around 76% and a specificity of around 86% leading to many cases of cervical cancer being missed or interpreted as negative.4-6

It is well accepted that the human papillomavirus (HPV) is a necessary causative factor in the development of cervical cancer. HPV deoxyribonucleic acid (DNA) is detectable in 99.7% of cervical cancer cases.7 Multiple randomized controlled trials and studies have shown that HPV DNA testing is significantly more sensitive than traditional cytology.8-10 In addition, studies have shown that a negative HPV test infers a longer low risk period of developing CIN2+/cancer than cytology.11 This makes HPV DNA tests an attractive possibility in cervical cancer screening.

However, HPV DNA tests have a lower specificity when compared to traditional liquid based cytology12, 13 because they look for the presence or absence of a gene. The non-selective nature of the DNA test predilects its inability to distinguish between transient infections, which clear on their own, and persistent infections, which are linked to potential malignant changes. It is thought that up to 90% of HPV infections regress spontaneously without treatment, most within the first twelve months.14 It is those persistent infections, in which the viral DNA is integrated, which pose the most risk of progression to carcinoma. The challenge lies in how to discriminate transient infections from persistent infections, thereby improving the HPV test specificity.

More recent insight into HPV’s lifecycle and the events that lead to cellular transformation have guided research in potential biomarkers that could provide both a sensitive and specific mechanism of identifying pre-malignant changes and improve
screening accuracy, either as an adjunct to current screening, or as a primary method. In 2006, the European Research Organization on Genital Infection and Neoplasia (EUROGIN) identified four main areas of research for appropriate biomarkers. One of the most promising advances identified was the detection of E6 and E7 mRNA.15

Two viral oncoproteins, E6 and E7, have been found to have well delineated roles in the neoplastic transformation of endocervical cells. Expression of E6 and E7 oncoproteins only occurs with persistent HPV infections and results in events leading to the degradation of the tumor suppressor gene products, P53 and pRb, and ultimately to an unregulated cell cycle.16, 17 This promising advance could lead to a more specific HPV test for detection of pre-malignant or malignant cervical lesions.

The APTIMA HPV (AHPV), which was recently approved by the FDA on October 28, 2011 and is now approved in 47 countries, tests for the E6/E7 mRNA of 14 high-risk HPV types. It is approved to run on Gen-Probe’s fully automated system decreasing interpretation variability. The FDA approved the use of AHPV as an adjunctive test to the pap in women 30 or older and for triage of ASCUS cytology in women 21 and older in accordance with the current cervical cancer recommendations based on the CLEAR trial, which analyzed 11 000 women presenting for routine screening at 18 US clinics.18

As new evidence becomes available and research guides evidence based medicine practices, it is likely that the face of cervical cancer screening will change over time. Randomized control trials have shown HPV testing to be a hopeful direction for the primary screening role when combined with reflex cytology for triage of positive results.12, 13, 19, 20 This reversal of the traditional roles provides the benefit of increased
sensitivity. A five year observational study of 331,818 women, 30 years or older, in the United States who were tested with both the HPV DNA test and liquid based cytology determined that based on the five-year incidence of CIN3 and cervical cancer, HPV testing followed by cytology triage would efficiently detect more cases of CIN3 and cancer than cytology alone. Preliminary models assessing cost effectiveness seem to support the use of HPV testing in cervical cancer screening as well. The question which remains is: would an HPV test, such as one that utilizes E6/E7 mRNA detection, provide both increased sensitivity and adequate specificity to warrant changing practice habits?

This systematic review addresses the question of whether the Aptima HPV mRNA test might offer the desired improved specificity while maintaining high sensitivity in comparison to HPV DNA testing or liquid based cytology, possibly guiding the role of this hopeful biomarker. E6/E7 mRNA tests, such as Aptima, could play a role in the screening of women for cervical neoplasms in both the industrialized and developing nations, representing the next step forward in diagnosis and prevention of cervical cancer.

METHODS

An exhaustive literature search was conducted in Medline, CINAHL, Web of Science, Google Scholar, and EBMRmultifile using the search terms *papillomaviridae, uterine cervical dysplasia, RNA, and sensitivity and specificity* in combination and alone. Scientific terms that were synonymous with these terms, including, *cervical intraepithelial neoplasm, uterine cervical neoplasm, and papillomavirus* were searched to prevent the omission of any relevant articles. The references of selected articles were
screened for the presence of any articles not produced in the original literature search. No limitations were placed on the search.

Articles not written in the English language were excluded, duplicates were removed and then titles and abstracts were screened for relevancy. Excluded were articles that assessed HPV screening in the HIV population, non-cervical methods of screening, screening conducted on self-collected samples, and those studies not utilizing both the APTIMA HPV mRNA assay and the Hybrid Capture II test. (See Figure 2.)

The articles reviewed were critically appraised using the GRADE approach to evaluate their validity. Each article was placed into a category, high, medium, low and very low, based on the quality of evidence.

RESULTS

Seven accuracy studies were included in this review based on the inclusion/exclusion criteria delineated in the methods section. Three prospective, blind comparison to a gold standard studies and four cohort studies with blind comparison to the gold standard. All studies included showed the Aptima HPV mRNA assay (AHPV) to have comparable sensitivity to Hybrid Capture II DNA test (HC2) and statistically better specificity for detection of clinically significant cervical lesions classified as CIN2+. Four of the studies compared the AHPV to both HC2 and liquid based cytology (LBC) and demonstrated AHPV and HC2 to have better sensitivity for CIN2+ than LBC.

Monsonego et al

Monsonego et al recently published a prospective study with a blind comparison to histology as the gold standard performed on a large screening based
population in Paris, France. From April 2008 to February 2009, women age 20-65 presenting for routine screening at seventeen gynecological offices were invited to participate in the study with a protocol in accordance to the Declaration of Helsinki and approved by the Independent Ethics Committee. Patient demographic data was evaluated and included. Exclusion criteria were set as prior hysterectomy, current pregnancy, or abnormal cytology results in the past six months. In total, 5006 women were enrolled with 525 women excluded: 3 for lack of consent, 53 for protocol violations, and 469 for invalid cytology results.23

The final set of eligible participants (n=4481) had an additional 52 women excluded due to absence of one or more of the HPV tests. This resulted in a total of 4429 women receiving a liquid based cytology sample collected with a Cervex-Brush and placed in PreservCyt medium. A ThinPrep liquid pap test along with both HC2 and AHPV tests were then performed.23

The LBC sample was analyzed at the Laboratoire Lavergne, Paris, France and classified using the 2001 Bethesda System by blinded cytopathologists. All abnormal results and a random 14% of normal results were blindly double-read by an independent external reviewer.23

The LBC sample was then divided into two equal portions and tested with both the HC2 and AHPV assays in alternating sequence in groups of 2500 according to manufacturer’s instructions at a central laboratory. All persons performing these tests were blinded to the LBC results.23

Women positive for any of the above tests and 14% of women negative for all three, selected via a random sample, were referred for colposcopy. For colposcopies in
which lesions were identified, biopsies were collected from areas of identified abnormalities as well as each quadrant of the transformation zone. Those women with a positive screening test but negative colposcopy received a biopsy at both 12 and 6 o’clock. No biopsy was performed on women who were negative for all three screening tests with a negative colposcopy. All biopsies were examined by a histopathologist, blinded to the HPV results at a central laboratory, and re-examined by an independent reviewer who was also blinded to the HPV results.  

Demographic differences and screening test results by age were assessed utilizing the Wilcoxon rank sum test and Fisher’s exact test. Sensitivity, specificity and predictive values were estimated using maximum likelihood adjusted for verification bias via extending the proposed method for verification-bias adjustment estimators by Zhou et al and Roldan-Nofuentes et al to accommodate all three screening tests. CIN2+ and CIN3+ were used as endpoints for calculations.

Monsenego et al determined that out of 4481 LBC cytology samples there were 723 women with normal cytology results via LBC who received histology results: 278 from the random negative sample and 445 who were positive for one or more of the HPV screening tests. Of these 723 cytological negative samples: 24 CIN2, 6 CIN3, and one adenocarcinoma in situ were identified. All 31 of these clinical diseases which cytology failed to detect were from the group screening positive for one of the HPV tests. They also determined that in women with abnormal cytology, approximately six colposcopies would need to be performed to find one CIN2+ lesion and approximately twenty-one to find one CIN3+ lesion.
In addition, they determined that both HC2 and AHPV performed with high
sensitivity in detection of CIN2+ clinical lesions (HC2 96.7% and AHPV 92.0%) and
were more sensitive than LBC (69.1% p<0.006). AHPV was significantly more specific
than HC2 in detection of CIN2+ lesions (91.8% vs. 86.4%, p<0.001) and similar in
specificity to LBC (91.8%). (See Table 1.)

Wu et al

Wu et al25 performed a prospective cohort study utilizing 2098 unscreened or
poorly screened women age 25-59 years from Shenzhen, China according to a protocol
approved by the institutional review board from the Peking University Shenzhen Hospital
and the Cleveland Clinic (Cleveland, Ohio). Exclusion criteria were set as prior
hysterectomy, current pregnancy, history of pelvic radiation, or cervical cancer screening
in the past three years. Demographic characteristics were collected and reported.25

Cervical specimens were collected in SurePath liquid for cytology and PreservCyt
medium for HPV testing via HC2 and AHPV. Three women with missing results and 95
women who were lost to follow up were excluded from the final data set of 2000 women.
Women positive for cytology or either of the HPV tests were referred for colposcopy and
biopsy of any area of abnormalities detected and at 2, 4, 8 and 10 o’clock positions of all
normal appearing quadrants followed by endocervical curettage.25

All tests were performed in accordance to manufacturer’s instructions. HC2 tests
were performed at the Royal Ladies Gynecology Clinic by individuals blinded to all other
test results. AHPV tests were performed by Gen-Probe at a remote site in a blinded
fashion. Cytology and pathology results were performed by specialists blinded to all
HPV test results at the Peking University Shenzhen Hospital in Shenzhen.25
Wu et al\textsuperscript{25} concluded that when the performance of all three screening mechanisms was evaluated using the histological diagnosis of CIN2+ as a clinical endpoint, the sensitivities of LBC, HC2, and AHPV were determined to be 66.7\%, 88.9\% and 100\%, (see Table 1) respectively, with a statistically significant difference between AHPV and LBC (p<0.004 by the McNemar test). In addition, it was noted that there were 15 cases of CIN3+ identified: 10/15 detected by LBC, 14/15 by HC2, and 15/15 detected by AHPV. The specificities of LBC, HC2, and AHPV were calculated at 95.5\%, 84.5\%, and 91.2\%, respectively. (See Table 1.) These results led them to draw a final conclusion that the high sensitivity and specificity of the Aptima test make this assay a candidate for use in primary screening for the detection of cervical disease noting that it can be run on Gen-Probe’s existing STD platform for \textit{Gonococcus} and \textit{Chlamydia}.\textsuperscript{25}

\textbf{Clad et al}

In the retrospective, referral based cohort conducted by Clad et al\textsuperscript{26} the sensitivity and specificity of AHPV, HC2, and LBC were determined, to compare each test’s ability to detect high-grade cervical lesions (CIN2+). In total, 492 specimens were collected from women referred to the Universitaets-Frauenklinik in Freiburg, Germany for colposcopy due to an abnormal screening pap. Samples were collected during colposcopy using ThinPrep in PreservCyt medium and stored for up to three years at room temperature. Exclusion criteria were identified as current pregnancy, missing histology results or inadequate specimens, and vaginal or vulvar dysplasia.\textsuperscript{26}

Colposcopies were performed by one physician and images of findings were digitally documented. Patients with positive findings were subjected to biopsy and later
treated according to standard of care. Patients with abnormal pap results but no visible lesions were subjected to loop electrosurgical excision procedure (LEEP). Those with a normal pap result and a normal colposcopy were not subjected to biopsy and considered “histology normal”.26

All conventional and LBC specimen evaluations were carried out at the Institute of Pathology – University of Freiburg, Germany and then residual samples stored for future HPV testing. The HC2 test and AHPV test were performed blinded to both the cytology and histology results at the University of Heidelberg, Germany and Gen-Probe Incorporated, San Diego, CA according to manufacturer’s instructions.26

Clad et al26 concluded that the sensitivity of the three screening mechanisms for detection of CIN2+ lesions, when calculated by comparison with histology, were LBC (84.90%), HC2 (91.30%), and AHPV (91.70%). The AHPV assay had the highest specificity at 75.00% followed by LBC 66.30%. (See Table 1.) It is noted that both AHPV and HC2 had a statistically significantly higher sensitivity (p=0.0041 for AHVP and p=0.0094 for HC2) than cytology. In addition, AHPV had a significantly higher specificity than HC2 (p<0.001) and cytology (p=0.0163) for the detection of CIN2+. 26

After the study was complete, CIN2 lesions were detected at the follow up of two patients with negative HC2, colposcopy, and cytology results but a positive AHPV test. In addition, one pregnant woman with an abnormal pap returned two months after delivery. In this woman, the AHPV test remained positive while her cytology and HC2 test returned to negative. An extensive CIN3 lesion was detected 2 years later.26

Overall, the conclusion was drawn that the AHPV assay is able to detect HPV high-risk mRNA in retrospective clinical LBC with high correlation to cervical disease.
In addition, the AHPV is significantly more sensitive and specific than cytology. Clad et al concluded that the AHPV assay performs better than HC2 and LBC in a referral-based population.26

**Ratnam et al**

Ratnam et al28 compared the AHPV and HC2 assays to histology in a referral population of women from five clinics in five providences across Canada for a longitudinal cohort study. Women aged 15 years and older with a cytological abnormality referred for colposcopy in the past two years who had not sought treatment were eligible for the referral group. In addition, another set of women presenting for routine screening were included.28

Upon enrollment, all women had a single cervical specimen collected with ThinPrep in PreservCyt medium, which was evaluated at the Public Health Laboratory, St John’s, Newfoundland, Canada using the 2001 Bethesda system. Residual fresh specimens were used for testing of AHPV and HC2 within two weeks of collection according to the manufacturer’s instructions. All researchers and individuals performing the tests were blinded to the results obtained from cytology, colposcopy, and histology.28

Colposcopy and, if indicated, biopsy were performed with histology being read by pathologists at each respective study site. Results of CIN2+ served as clinical endpoint for data analysis. All pathologists were blinded to HPV results.28

Data were analyzed to determine the clinical performance of each test using histology as gold standard. Sensitivity and specificity were calculated using contingency tables and the 95% confidence intervals computed using the binomial method.
McNemar’s chi-square test was used to test the differences between sensitivity and specificities.\textsuperscript{28}

Ratnam et al\textsuperscript{28} determined that AHPV had a sensitivity of 96.3\% for CIN2+ compared to HC2 which had a sensitivity of 94.3\% with no statistical significance between the two modalities. AHPV had a specificity of 43.2\% compared to HC2’s specificity of 38.7\% (p<0.05). (See Table 1.) In addition, AHPV detected all 13 cases of cervical cancer, whereas HC2 only detected 12/13. AHPV would reduce colposcopy referrals by approximately 6.0\% if used as adjunct to ASCUS cytology in the place of HC2. This study concluded that the AHPV test has the potential to serve as a feasible option for both primary cervical cancer screening and the triage of borderline cytology diagnoses.\textsuperscript{28}

\textbf{Dockter et al}

Dockter et al\textsuperscript{29} presents a study to assess the clinical performance of AHPV compared to HC2 in the detection of high-risk HPV and relevant disease (CIN2+) in 800 specimens collected with ThinPrep pap in PreservCyt medium from women referred for colposcopy in Paris, France. Colposcopies were performed and biopsies obtained from any visible lesions. No biopsies were collected from those subjects with a negative colposcopy and considered disease negative.\textsuperscript{29} All testing was performed in a blinded fashion (J. Dockter, e-mail communication, January 26, 2012).

The AHPV was run on both the direct tube system (DTS) and the fully automated TIGRIS system according to manufacturer’s instructions. The HC2 was also run from the same clinical specimens in accordance with manufacturer’s instructions. The sensitivity, specificity, positive predictive value and negative predictive value were
determined for both the AHPV and the HC2 using histology results of CIN2+ and CIN3+ as clinical endpoints. 95% Confidence intervals were calculated with the Score method and McNemar chi-square test was utilized to determine p values.  

AHPV assay was determined to have a clinical sensitivity for detection of CIN2+ of 90.8% (84.9-94.5) on both the DTS and the TIGRIS system with no statistically significant difference compared to the 95.0% (90-97.6) for the HC2 test with a p>0.05, while the specificity of the AHPV at 55.4-56.2% (51.4-60.1) was statistically significantly more specific than the HC2’s performance of 47.4% (43.5-51.4) p<0.0001. (See Table 1.) The observed decrease in specificity of the HC2 test equates to as many as 54 colposcopy referrals in false positive specimens with normal colposcopy findings compared to the AHPV assay. Overall, based on the study, Dockter et al believe that the AHPV assay was both highly sensitive and specific for detection of high-grade cervical lesions in the tested referral population.  

**Szarewski et al**

This cohort study was conducted by Szarewski et al to compare the sensitivity and specificity of several tests including the AHPV and HC2 for detection of high-grade CIN2+ as diagnosed by biopsy and histopathology in 999 women referred for colposcopy for abnormal cytology between August 2005-January 2007 in London, England. Exclusion criteria were set as current pregnancy, previous treatment of CIN, and hysterectomy. Non-eligible women and excluded women were identified clearly in the report. The study protocol was reviewed by the local research ethics committee.  

Before colposcopy was performed, a cervical sample was obtained with the ThinPrep system in PreservCyt medium, which was used for LBC and HPV testing. The
LBC samples were analyzed at The Doctors’ Laboratory. HPV testing was then performed according to manufacturer’s instructions at the Cancer Research UK except for the AHPV test, which was carried out by the Gen-Probe. All laboratories performing molecular testing were blinded to the cytology and histopathology results. All histopathology results were reviewed locally by Dr. Hilary Buckley or Dr. Christine Bergson, who were both blinded to all HPV test results.24

Statistical analyses were made using Stata 9.2 from Stata Corp. The gold standard of histologically confirmed CIN2+ was used for clinical endpoint calculations of sensitivity, specificity, positive predictive value, and negative predictive value for each test along with 95% exact binomial confidence intervals. Analyses indicated that the AHPV had a sensitivity of 95.2% for CIN2+ whereas the HC2 had a sensitivity of 99.6%, but that the AHPV had the highest specificity at 42.2% as opposed to HC2’s specificity of 38.8%. The referral pap cytology had a sensitivity of 93.1% and a specificity of 17.8% for CIN2+ lesions while the cytology collected on the day of colposcopy had a sensitivity of 92.3% and a specificity of 60.8%.24 (See Table 1.)

It was concluded that by comparing a wide range of adjunctive tests on the same samples, with histology as the gold standard, that both the AHPV and the HC2 had high sensitivity. Furthermore, they are unlikely to miss significant clinical disease, but the AHPV assay had better specificity correlating to fewer false positives and unnecessary follow-up.24

Reuschenbach et al

In this cross-sectional cohort by Reuschenbach et al,27 the AHPV and HC2 tests were evaluated on 275 liquid based cytology specimens from women who attended the
A total of 316 women were enrolled and based on exclusion criteria of invalid results in one or more of the tests, pregnancy, and vulvar or vaginal carcinomas; 275 were included in the final study. The median age of those enrolled was 36 years old. Colposcopy was performed on all women during which cytology samples were collected and placed in PreservCyt medium and biopsies taken from all but 38 women. All tests were performed in accordance to manufacturer’s instructions. The gold standard of histologically confirmed CIN2+ was used as clinical endpoint for statistical analysis.27

Rueschenbach et al27 determined that for the cohort of women selected, the sensitivity of AHPV was 88.4% (82.3-92.7) vs. 91.5%(85.8-95) for HC2. In addition, they determined that AHPV had better specificity than HC2 for detection of CIN2+, 63.4% vs. 71.2% respectively.27 (See Table 1.)

It was concluded that AHPV had a similar sensitivity for detection of CIN3+ and slightly lower for CIN2+ when compared to HC2, but much higher specificity for detection of both endpoints. Thus, an enhanced diagnostic profile was observed by the use assays targeting oncogenic proteins, E6 and E7, such as AHPV.27

DISCUSSION

This systematic review clearly shows that the AHPV assay could play a promising role in the future of cervical cancer detection. A summary of findings is presented in Table 1, which shows AHPV to have a similar sensitivity to the widely accepted HC2 DNA test in both the screening population23, 25 and the referral population.24, 26-28, 30 AHPV sensitivity ranged from 88.4%-92.0% (95%CI, 82.3-100) compared to HC2 88.9%-99.6% (95% CI, 70.8-100) across the seven analyzed studies.23-30 In Monsenego et
al, it was noted that of a subset of 15 CIN3 lesions, the AHPV detected 15/15 compared to 14/15 for HC2 and only 10/15 for LBC. This is consistent with other studies, which have shown HPV mRNA tests to have a sensitivity similar to HPV DNA tests while offering improved specificity. Most interestingly, AHPV showed improved specificity over the HC2 test in all seven studies included in this review, further strengthening the test’s future role in medical decision-making. AHPV had a specificity ranging from 91.2%-91.8% with narrow 95% confidence intervals between 89.8-92.6 for the large screening-based populations of Monsenego et al and Wu et al. AHPV also had stronger specificities in the range of 42.2%-75.0% (95% CI, 38.4-80.9) for the referral based populations of the other included studies. Overall, these comparisons show that AHPV mRNA assay performs strongly when compared to the HC2 HPV DNA test. The increase in specificity over HPV DNA testing equates to fewer false positives, saving both the cost of workup and the harms related to unnecessary procedures.

In four of the studies reviewed, the AHPV assay was compared to both HC2 and liquid based cytology. These studies concur with the previous meta-analysis, comparing HC2 DNA testing to LBC, that HPV DNA testing is more sensitive than LBC, but lacks specificity. Interestingly, two studies point to the AHPV mRNA assay and LBC having similar specificities (91.8% vs 92.7% in Monsenego et al) and (95.5% vs. 91.2% in Wu et al) in the screening-based population. In fact, the confidence intervals overlap in both of these studies precluding any determination of significance of the noted difference. Three of the studies using referral-based populations did not report the sensitivity/specificity calculation for LBC. Two studies, Clad et al and Szarewski
et al.,24 reported the AHPV to have an even better specificity when compared to LBC for detection of CIN2+ lesions in the referral population.

Likelihood ratios were calculated (see Table 3) and demonstrated that AHPV has a LR+ greater than 10 in both the large population based studies of Monsonego et al23 and Wu et al25 making it a viable test to rule cervical neoplasm in, as well as a LR- ratio of less than 0.1 making it viable to also rule out cervical neoplasm. This strengthens the evidence to support the use of AHPV mRNA tests in the screening population. As seen in Table 3, AHPV performed quite well compared to HC2 and LBC in all of the studies included with larger LR+ correlating to stronger diagnostic ability.

Likelihood ratios were graphed34 for all studies included in order to better compare the tests overall predictive value. (See Figure 3.) It can be concluded that AHPV performed superior to LBC in three of the four studies21,23,24 which reported LBC sensitivities and specificities and was better at detecting the presence of disease in the other study, Wu et al22. AHPV also performed superior to HC2 in three of the studies22,23,25 and better at detecting the absence of disease in the other four20,21,24,26.

Limitations

Limitations of all included studies were reviewed. (See Table 2.) Both population-based studies23,25 were well done in a prospective manner: providing adequate sample numbers, control groups, comparison against the gold standard of histology, and proper blinding. The referral-based studies varied in strength of design. All studies were subject to indirectness as they all utilized the outcome of CIN2+. This is a minor area of indirectness as CIN2+ is also the threshold for medical intervention and therefore still a patient important outcome.
Monsenego et al\textsuperscript{23} had a sample size of 4481 women presenting for routine screening with a randomly selected 14\% of negative samples (491) used as the control group. A larger control group would have been preferential to maintain balance in the two groups. All results were adjusted for verification bias and mechanisms for multiple reviewers and resolution of discordant results were made transparent. Comparisons were made utilizing the gold standard of biopsy and histological diagnosis. No areas of bias could be identified.

Similarly, Wu et al\textsuperscript{25} utilized selection based on routine screening, which allowed for a representative subject group as identified in the demographic data provided. The total subject pool was comprised of 2098 women who received biopsies in five cervical areas if positive for any of the three screening tests. Proper blinding was maintained. Those women who were negative for cytology and both HPV tests were considered the control. While it would have been preferential to have a portion of these women undergo biopsy as the gold standard, this does not greatly influence the overall strength of the data collected. In addition to direct statistical comparisons, the partial areas under the receiver operating curves were compared.

Clad et al\textsuperscript{26} performed a study utilizing a referral-based population, inclusion and exclusion criteria were identified and blinding was adequately performed. This group of women may not be representative of the average woman making any conclusions about the sensitivity and specificity of the tests for screening impossible. But, this study does allow for the analysis of the performance in a triage scenario and also allows for a larger variation of disease to be represented. In addition, only 424 specimens were analyzed. A larger sample size would have made the evidence stronger. Three main limitations were
identified: 1) specimens were stored at room temperature for up to three years before testing but not a consistent time frame from specimen to specimen 2) no biopsies were taken from patients with a normal colposcopy and a normal cytology screening 3) HPV tests were run later, no colposcopies were done for those specimens that were only positive by HPV.

Ratnam et al\textsuperscript{28} was overall fairly well conducted but did have some inconsistencies identified, such as not all subjects had histology results and those who were HPV positive but cytology negative did not receive a colposcopy due to the design of the study. In addition, the outcome of CIN2+ was used, which as discussed above, is only a minor area of indirectness.

In the study performed by Dockter et al\textsuperscript{29}, an area of bias identified was that J. Dockter is employed by Gen-probe. The outcome of CIN2+ was utilized and those with negative colposcopy were not subjected to biopsy. Szarewski et al\textsuperscript{24} also faced similar limitations. Reuschenbach et al\textsuperscript{27} also utilized CIN2+ as an outcome resulting in minor indirectness as discussed above, in addition after exclusion criteria were applied the total sample size studied was only 275 participants resulting in wider confidence intervals and an identified area of possible imprecision.

The quality of each study was assessed utilizing the GRADE assessment tool. (See Table 2.) Monsonego et al\textsuperscript{23} and Wu et al\textsuperscript{25} remained at high quality while the other included studies were downgraded to moderate\textsuperscript{24,26,28} or low\textsuperscript{27,29} based on limitations identified. Overall the quality of evidence of this body of literature is moderate. (See Table 2.) Showing that further research is needed, but unlikely to drastically change
confidence in these results. Instead, further research will more fully support and refine
the evidence for the use of HPV screening in the detection of cervical neoplasms.

Conclusions
Reviews such as this illuminate the need for further research to determine both the
long-term viability and cost effectiveness of utilizing HPV mRNA tests, such as AHPV,
in the detection of cervical lesions in a primary screening role for cervical cancer.
Randomized controlled trials as well as longitudinal studies that evaluate the overall
effect on, not just the outcome of CIN pre-malignant lesions, but on the actual morbidity
and mortality of cervical carcinoma are needed. Also promising, is the possibility of
utilization of self collected specimens for community based screening programs in areas
of the world with the highest cervical cancer mortality rates and the lack of infrastructure
to support traditional screening programs.

These findings are relevant in our decision making in order to provide the best
patient care, by practicing evidence based medicine, we are likely to change the current
practice of using the HC2 HPV DNA test for triage of borderline cytology, as well as in
HPV co-testing of women age 30 and older, to the use of AHPV mRNA assay. Both the
AHPV and HC2 tests are approved in those situations, but the AHPV provides both
improved specificity while maintaining high sensitivity.
### Table 1 Summary of Findings

<table>
<thead>
<tr>
<th>STUDY:</th>
<th>Study Type</th>
<th>Population</th>
<th>Age Range</th>
<th>Comparison</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>Conclusions:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Monsenego, et al</strong>&lt;sup&gt;23&lt;/sup&gt;</td>
<td>Accuracy Study – Prospective, Blind Comparison to Gold Standard</td>
<td>4429 ♀ presenting for routine screening at gynecology office in Paris, France between 8/08-2/09</td>
<td>20-65</td>
<td>AHPV mRNA, HC2 DNA, and thin prep pap cytology vs. histology</td>
<td>92.0% (86.4-97.6)</td>
<td>96.7% (92.6-100)</td>
<td>69.1% (60.0-78.1)</td>
</tr>
<tr>
<td><strong>Wu, et al</strong>&lt;sup&gt;25&lt;/sup&gt;</td>
<td>Accuracy Study – Prospective, Blind Comparison to Gold Standard</td>
<td>2000 ♀ from medically underserved Luohu district in Shenzhen, China</td>
<td>25-59</td>
<td>AHPV mRNA, HC2 DNA, and thin prep cytology vs. histology</td>
<td>100% (87.2-100)</td>
<td>88.9% (70.8-97.6)</td>
<td>66.7% (46.0-83.5)</td>
</tr>
<tr>
<td><strong>Clad, et al</strong>&lt;sup&gt;26&lt;/sup&gt;</td>
<td>Accuracy Study- Retrospective, Blind Comparison to Gold Standard</td>
<td>385 ♀ referred to Universitaets-Frauenklinik Freiburg, Germany colposcopy</td>
<td>NR</td>
<td>AHPV mRNA, HC2 DNA, and thin prep cytology vs. histology</td>
<td>91.7% (87.6-94.5)</td>
<td>91.3% (87.1-94.2)</td>
<td>84.9% (80.0-88.9)</td>
</tr>
<tr>
<td><strong>Ratnam, et al</strong>&lt;sup&gt;28&lt;/sup&gt;</td>
<td>Referral based longitudinal cohort</td>
<td>1,418 referral cases and 1,373 routinely screened ♀ from 5 centers in 5 provinces across Canada</td>
<td>16-81</td>
<td>AHPV mRNA, HC2 DNA vs. histology</td>
<td>96.3% (94.4-98.2)</td>
<td>94.3% (92.0-96.6)</td>
<td>NR</td>
</tr>
<tr>
<td><strong>Dockter, et al</strong>&lt;sup&gt;29&lt;/sup&gt;</td>
<td>Referral based cohort</td>
<td>800 liquid pap specimens collected from ♀ in Paris who were referred for colposcopy for abnormal screening cytology</td>
<td>NR</td>
<td>AHPV mRNA, HC2 DNA vs. histology</td>
<td>90.8% (84.9)</td>
<td>95.0% (90.1-97.6)</td>
<td>NR</td>
</tr>
<tr>
<td><strong>Szarewski, et al</strong>&lt;sup&gt;24&lt;/sup&gt;</td>
<td>Referral based cohort</td>
<td>953 ♀ referred for colposcopy in London between August 2005-January 2007</td>
<td>Median age 29.9 range not reported</td>
<td>AHPV mRNA, HC2 DNA, thin prep pap cytology vs. histology</td>
<td>95.2% (92.0-97.4)</td>
<td>99.6% (98.0-100.0)</td>
<td>93.4% (89.8-96.0)</td>
</tr>
<tr>
<td><strong>Reuschenbach, et al</strong>&lt;sup&gt;27&lt;/sup&gt;</td>
<td>Referral based cross-sectional cohort</td>
<td>316 ♀ who attended University Hospital of Freiburg, Germany for abnormal cytology or follow-up for treatment of dysplasia between 2005-2007</td>
<td>Median age 36, range not reported</td>
<td>AHPV mRNA, HC2 DNA, thin prep pap cytology vs. histology</td>
<td>88.4% (82.3-92.7)</td>
<td>91.5% (85.8-95.1)</td>
<td>NR</td>
</tr>
</tbody>
</table>

AHPV = APTIMA HPV mRNA test  
HC2 = Hybrid Capture II DNA test  
LBC = liquid based cytology  
NR= not reported  
CIN= cervical intraepithelial neoplasm
Table 2 Quality of Evidence

<table>
<thead>
<tr>
<th>Study</th>
<th>Study Design</th>
<th>Risk of Bias</th>
<th>Inconsistency</th>
<th>Indirectness</th>
<th>Imprecision</th>
<th>Sample Size</th>
<th>Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monsenego, et al&lt;sup&gt;23&lt;/sup&gt;</td>
<td>Accuracy Study – Prospective, Blind Comparison to Gold Standard</td>
<td>No areas of bias identified</td>
<td>No inconsistencies</td>
<td>Minor area of indirectness&lt;sup&gt;1&lt;/sup&gt;</td>
<td>No serious imprecision</td>
<td>4429</td>
<td>⊕⊕⊕⊕ Ultra High</td>
</tr>
<tr>
<td>Wu, et al&lt;sup&gt;25&lt;/sup&gt;</td>
<td>Accuracy Study – Prospective, Blind Comparison to Gold Standard</td>
<td>No areas of bias identified</td>
<td>No inconsistencies</td>
<td>Minor area of indirectness&lt;sup&gt;1&lt;/sup&gt;</td>
<td>No serious imprecision</td>
<td>2000</td>
<td>⊕⊕⊕⊕ High</td>
</tr>
<tr>
<td>Clad, et al&lt;sup&gt;26&lt;/sup&gt;</td>
<td>Accuracy Study - Retrospective, Blind Comparison to Gold Standard</td>
<td>No areas of bias identified</td>
<td>Minor inconsistencies&lt;sup&gt;2, 3&lt;/sup&gt;</td>
<td>Minor area of indirectness&lt;sup&gt;1&lt;/sup&gt;</td>
<td>No serious imprecision</td>
<td>385</td>
<td>⊕⊕ Moderate</td>
</tr>
<tr>
<td>Ratnam, et al&lt;sup&gt;28&lt;/sup&gt;</td>
<td>Accuracy Study- Blind Comparison to Gold Standard</td>
<td>No areas of bias identified</td>
<td>Minor inconsistencies&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Minor area of indirectness&lt;sup&gt;1&lt;/sup&gt;</td>
<td>No serious imprecision</td>
<td>1,418 referral cases, 1,373 screening</td>
<td>⊕⊕ Moderate</td>
</tr>
<tr>
<td>Dockter, et al&lt;sup&gt;29&lt;/sup&gt;</td>
<td>Accuracy Study- Blind Comparison to Gold Standard</td>
<td>Potential areas of bias identified&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Minor inconsistencies&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Minor area of indirectness&lt;sup&gt;1&lt;/sup&gt;</td>
<td>No serious imprecision</td>
<td>800</td>
<td>⊕⊕ Low</td>
</tr>
<tr>
<td>Szarewski, et al&lt;sup&gt;24&lt;/sup&gt;</td>
<td>Accuracy Study- Blind Comparison to Gold Standard</td>
<td>Possible areas of bias identified&lt;sup&gt;1&lt;/sup&gt;</td>
<td>No inconsistencies</td>
<td>Minor area of indirectness&lt;sup&gt;1&lt;/sup&gt;</td>
<td>No serious imprecision</td>
<td>953</td>
<td>⊕⊕ Moderate</td>
</tr>
<tr>
<td>Reuschenbach, et al&lt;sup&gt;27&lt;/sup&gt;</td>
<td>Accuracy Study- Blind Comparison to Gold Standard</td>
<td>No areas of bias identified</td>
<td>Minor inconsistencies&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Minor area of indirectness&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Small amount of imprecision noted&lt;sup&gt;6&lt;/sup&gt;</td>
<td>316</td>
<td>⊕⊕ Moderate</td>
</tr>
</tbody>
</table>

OVERALL QUALITY OF EVIDENCE

CONCLUSION:

⊕⊕⊕⊕ Ultra High

1 Surrogate outcome of CIN2+ was used, considered a minor indirectness as this is also the threshold for medical intervention, which is a patient important outcome.

2 Biopsy specimens were not taken from those with patients with normal colposcopy and cytology.

3 The HPV testing was done at a later date and biopsies were not done of those women who were only positive for one of the HPV tests.

4 J. Dockter employed by Gen-probe

5 A. Szarewski is on the advisory board of Gen-probe, consultant for Roche, and speaker for Qiagen. The AHPV test was run by Gen-probe for all samples.

6 After set exclusion criteria were applied, n=275, 95%CI were wider than observed in other studies.

Table 3 Likelihood Ratios

<table>
<thead>
<tr>
<th>Study</th>
<th>+ Likelihood Ratio AHPV</th>
<th>- Likelihood Ratio AHPV</th>
<th>LBC</th>
<th>LBC</th>
<th>HC2</th>
<th>LBC</th>
<th>HC2</th>
<th>LBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monsenego, et al&lt;sup&gt;23&lt;/sup&gt;</td>
<td>11.20</td>
<td>.087</td>
<td>7.11</td>
<td>.038</td>
<td>.336</td>
<td>8.53</td>
<td>.038</td>
<td>.336</td>
</tr>
<tr>
<td>Wu, et al&lt;sup&gt;25&lt;/sup&gt;</td>
<td>11.36</td>
<td>.000</td>
<td>5.74</td>
<td>.130</td>
<td>.349</td>
<td>14.82</td>
<td>.130</td>
<td>.349</td>
</tr>
<tr>
<td>Clad, et al&lt;sup&gt;26&lt;/sup&gt;</td>
<td>3.67</td>
<td>.111</td>
<td>2.34</td>
<td>.143</td>
<td>.228</td>
<td>2.52</td>
<td>.143</td>
<td>.228</td>
</tr>
<tr>
<td>Ratnam, et al&lt;sup&gt;28&lt;/sup&gt;</td>
<td>1.70</td>
<td>.086</td>
<td>1.54</td>
<td>.147</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Dockter, et al&lt;sup&gt;29&lt;/sup&gt;</td>
<td>2.07</td>
<td>.164</td>
<td>1.81</td>
<td>.105</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Szarewski, et al&lt;sup&gt;24&lt;/sup&gt;</td>
<td>1.65</td>
<td>.114</td>
<td>1.39</td>
<td>.014</td>
<td>.437</td>
<td>1.10</td>
<td>.014</td>
<td>.437</td>
</tr>
</tbody>
</table>
Figure 1: Incidence of Cervical Cancer World Wide

World age-standardized incidence rates of cervical cancer

ASR, age-standardized incidence rate; Rates per 100,000 women per year.

Figure 2: Search Methodology

Initial Literature Search

First Layer Exclusion

Screened by Title/Abstract for Relevancy (n=226)

Not in English Language (n=4)

Duplicates Removed (n=59)

Full Text Review

Full Text Articles Reviewed (n=36)

Not relevant based on Title/Abstract (n=18)

Final Selection

Articles Included in Systematic Review (n=7)

Articles Excluded based on Exclusion Criteria (n=31)

Articles Excluded (n=31)
Figure 3: Likelihood Ratio Graphs

Monsonego, et al

Wu, et al

Dockter, et al

Clad, et al

Ratnam, et al

Szarewski, et al

Reuschenbach, et al

Likelihood ratios graph: regions of comparison

Recreated from Biggerstaff

Key:

AHPV ρ+
AHPV ρ-
HC2 ρ+
HC2 ρ-
LBC ρ+
LBC ρ-
References


