



TITLE: Human Papillomavirus Testing in Cervical Cancer Screening

AUTHOR: Jeffrey A. Tice, MD
Assistant Adjunct Professor of Medicine
Division of General Internal Medicine
Department of Medicine
University of CA, San Francisco

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HUMAN PAPILLOMAVIRUS TESTING IN CERVICAL CANCER SCREENING

INTRODUCTION

The California Technology Assessment Forum is requested to review the scientific evidence for the use of human papillomavirus (HPV) testing to screen for cervical cancer.

BACKGROUND

Squamous cell cancer of the cervix will be diagnosed in approximately 12,200 US women in 2003 and 4,100 women will die from cervical cancer. Incidence in the US population has fallen from 14.2 cases per 100,000 women in 1973 to 7.8 case per 100,000 women in 1994. With this decrease in incidence, in 2003 cervical cancer dropped off of the list of the top 10 leading causes of cancer death for women in the US. (American Cancer Society 2003). Cervical cancer screening with cytology using the Papanicolaou (Pap) test is thought to be primarily responsible for the decreasing incidence of cervical cancer. International observational studies have consistently found that the incidence of cervical cancer falls by 60-90% within 3 years of widespread adoption of Pap testing in a population. (1986; Sasieni *et al.* 1996). There have been no randomized clinical trials of Pap tests, but the observational evidence is strong and consistent enough to obviate the need for such trials.

The success of cervical cancer screening programs reflects the fact that cervical cancer has a long pre-invasive phase lasting typically over 10 years. Cervical cytology screening can identify the pre-invasive disease and there is effective treatment for the pre-invasive disease. Furthermore, the long pre-malignant phase allows repeated tests to reduce the impact of a single false-negative test. The purpose of screening is not only to detect cancer, but also to identify and remove high-grade lesions and thus prevent potential progression to cervical cancer.

The system for reporting cervical cytology results has undergone multiple changes since the original guidelines proposed by Papanicolaou. The most widely used approach in the United States is the Bethesda System that was most recently updated in 2001. The range of findings and usual histologic equivalents are listed in Table 1.

Table 1: Classification of cervical squamous cell pathology

Cytology (Papanicolaou test)	Histology (Biopsy)
Negative	Normal
Atypical squamous cells – undetermined significance ASC-US	Variable
Low-grade squamous intra-epithelial lesion (LSIL)	Cervical intra-epithelial level, grade 1 (CIN 1)
High-grade squamous intra-epithelial lesion (HSIL)	Cervical intra-epithelial level, grade 2 (CIN 2)
Squamous cell carcinoma	Carcinoma in situ (CIN 3) Invasive carcinoma

In the United States, over half of cervical cancer is diagnosed in women who have never been screened or who have not been screened in the five years prior to diagnosis (Janerich *et al.* 1995; Womack *et al.* 1998). An additional 11% had abnormalities identified on the preceding Pap smear, but were not properly followed up. In only 7% of the cases of cervical cancer, were abnormalities present on the slide, but not detected by the screening test. In 25% of the cases, women had a normal pap within three years of being diagnosed with cervical cancer.

Current recommendations are for women to have cervical screening performed annually. After age 30, women who have had three consecutive negative Pap tests may be screened every two to three years. Observational data with inconsistent methodology have suggested that the relative risk associated with screening every two years is twice that of annual screening and that screening every three years triples the risk of invasive cervical cancer (1986; Lynge *et al.* 1986; Shy *et al.* 1986). However, the absolute risk is very low. Unfortunately, most of the studies do not report absolute risk. Data from one large prospective cohort study in the United States (n=128,805) were used to calculate the absolute risk of developing high grade cytological abnormalities within three years of a normal Pap test (Sawaya *et al.* 2000). The risks were 25/10,000 for women screened annually, 29/10,000 for women screened every two years, and 33/10,000 for women screened every three years. These differences were not statistically significant. A recent case-control study of invasive cervical cancer within a large HMO with over one million women estimated that the absolute risk of developing invasive cervical cancer within three years following three or more normal Pap tests to be less than 5/100,000 women (Miller *et al.* 2003). A second large study of over 900,000 women estimated the absolute risk of cervical cancer within three years after three negative Pap tests. For women who continued annual Pap testing, the incidence of cervical cancer would be 2/100,000 among women 30 to 44 years of age, 1/100,000 among women 40 to 59 years of age, and 1/100,000 among women 60 to 64 years of age (Sawaya *et al.* 2003). For women who were screened three years after the last negative Pap test, the incidence of cervical cancer would be 5/100,000 among women 30 to 44 years of age, 2/100,000 among women 40 to 59 years of age, and 1/100,000 among women 60 to 64 years of age. The authors estimate preventing one case of cervical cancer would require an

average of 69,665 Pap tests and 3,861 colposcopic examinations in women 30 to 44 years old and approximately three times that number for women 45-59 years old.

Systematic reviews suggest that the sensitivity of conventional cervical cytology is only 50-75% (Fahey *et al.* 1995; Nanda *et al.* 2000). This is thought to be due to two reasons: sampling error (cells from the abnormal area of the cervix were not sampled during the collection of the specimen) and detection error (abnormal cells collected on the specimen, but not identified) (Janerich *et al.* 1995; Hildesheim *et al.* 1999). Currently, the US Government has mandated a 10% repeat reading of all Pap smears to minimize detection error. New technologies, such as thin layer cytology and computerized re-screening of negative smears have also been introduced to reduce detection error. However, reducing detection error is unlikely to have a large impact on cervical cancer rates as only 7% of cases of cervical cancer were preceded by Pap tests which were misread as negative. HPV testing has been proposed as a way to decrease both the sampling and detection errors and thus may have a larger impact on reducing cervical cancer incidence than the other technologies.

The primary risk factor for cervical cancer is infection with oncogenic HPV types, which will be described in greater detail below. As HPV infection is transmitted sexually, factors related to sexual behavior have been associated with increased risk including number of sexual partners and age of first intercourse. Cigarette smoking has also consistently been correlated with a 2-4 fold increased risk of cervical cancer and appears to decrease clearance of HPV infections (Giulian *et al.* 2002; Biemelt *et al.* 2003; Warzecha *et al.* 2003). In the United States, the highest incidence and prevalence of HPV infection is in women under age 25 (Koutsky 1997).

It is now accepted that almost all squamous cell carcinoma of the cervix is caused by infection with one of 18 types of human papillomavirus (Munoz *et al.* 2003). Studies have identified HPV DNA in 95-100% of squamous cell cancers of the cervix and in 75-95% of high grade CIN lesions (Bosch *et al.* 1995; Jacobs *et al.* 1997; Walboomers *et al.* 1999; Munoz *et al.* 2003). More than 100 types of HPV have been identified, but most are not associated with cervical cancer. The best-characterized types associated with cervical cancer are Types 16 and 18 (Schiffman *et al.* 1995). Other HPV types that are considered high risk include types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. The key viral genes leading to oncogenesis are E6 and E7. They encode proteins that bind to and inactivate tumor suppressor proteins (Rb and p53) in the host genome. Viruses with E6 and E7 proteins that do not inactivate these proteins are not associated with cervical cancer (Park *et al.* 1995).

The natural history of HPV infection is complicated. Most women who become infected with HPV clear the infection and have no cytologic abnormalities. In a cohort study of women in college in the United States, 50% of HPV infections resolved within eight months to two years after testing positive, only 9% of the women continued to be positive for the same HPV type (Ho *et al.* 1998). The presence of intact viral particles within the cell may cause a characteristic peri-nuclear clearing called koilocytosis. Koilocytosis is a defining characteristic of LSIL on cytology and

CIN 1 on histology. Given the natural history of HPV infection, it is not surprising that the majority of cases of LSIL resolve without treatment. Development of cervical cancer almost always requires integration of the viral DNA into the host genome. Without integration, low-grade epithelial changes may be observed, but high-grade dysplasia and cancer are rarely observed (Hildesheim *et al.* 1994; Ho *et al.* 1998; Moscicki *et al.* 1998). Viral integration usually disrupts an important viral gene E2. Thus high-grade lesions usually do not shed viral particles and do not exhibit koilocytosis. It is estimated that about 60% of untreated high-grade lesions (HSIL, CIN 2 or 3) will progress to invasive cancer. The time from viral infection to oncogenesis is not known. Natural history studies suggest an orderly progression from infection to low grade epithelial changes to high grade changes over a period of many years.

Based on a large randomized clinical trial (Schiffman *et al.* 2000a; Solomon *et al.* 2001; Schiffman *et al.* 2003) guidelines now recommend that most women who have ASC-US on liquid-based Pap testing be tested for HPV. Women with ASC-US (n=3488) were randomized to immediate colposcopy, repeat Pap testing, or HPV testing using the Hybrid Capture II method (HC II, see below). Women positive for HPV or HSIL on repeat Pap testing were sent for colposcopy. All women had colposcopy at 24 months. Using a positive HPV test as the threshold for colposcopy, 56% of women were referred and 92% of the women with CIN 3 were identified. This approach was as sensitive as repeat Pap testing with referral of patients with ASC-US for colposcopy and fewer women required colposcopy.

HPV testing

Hybrid Capture II (HC II)

The standard Hybrid Capture II test detects the presence of 13 types of HPV that have been associated with cervical cancer. There is an extended version that detects an additional five HPV types that are less prevalent and lower risk, but this HC II test has not been evaluated in the large clinical trials. The high risk HPV test contains whole genomic RNA probes that detect HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 59, and 68. The probes bind complementary DNA from cervical cells (RNA-DNA complex = "hybrid"). The complexes are detected using an enzyme-linked chemiluminescent assay that uses signal amplification to achieve sensitive detection without DNA amplification. This reduces the risk of interference from materials present in the clinical preparation, which can be a problem for methods that depend on PCR target amplification. The recommended threshold for calling a test positive is 1 pg/ml, which corresponds to approximately 5000 HPV genomes per test well. The test can be performed on residual cells from a liquid based cytology specimen or from a specimen collected using a cervical brush placed in transport medium specifically for the HPV test.

Polymerase Chain Reaction

Other tests available for the detection of high-risk HPV types are based on PCR technology. They are highly sensitive for viral detection and can identify the specific viral type responsible for the infection as well as quantify the viral load. These tests use enzymatic amplification of HPV DNA to allow the detection of very low levels of HPV

infection. The test has excellent test characteristics in appropriately equipped and experienced laboratories. However, contamination with previously amplified material can lead to false positives and there is currently no standardization between laboratories. The most common PCR systems used in clinical studies are those based on the GP5+/6+ primers (Jacobs *et al.* 1997; Laconi *et al.* 2001) and the MY09/MY11 primers (Tachezy *et al.* 1994; Perrons *et al.* 2002). Because of intellectual property issues, no PCR-based test for HPV has been developed commercially and the FDA has not approved any PCR-based detection systems for use in the United States. This may change as soon as the *Institut Pasteur* has recently transferred its HPV intellectual property to F. Hoffmann-La Roche Ltd.

TECHNOLOGY ASSESSMENT (TA)

TA Criterion 1: The technology must have final approval from the appropriate government regulatory bodies.

On March 31, 2003 the FDA Center for Devices and Radiological Health (CDRH) approved a PMA supplement to provide for commercial distribution of the Digene Hybrid Capture® 2 (HC2) High-Risk HPV DNA Test (Digene Corporation, Gaithersburg, MD) as modified in accordance with conditions. The CDRH noted that “this device is indicated for:

1. To screen patients with ASCUS (atypical squamous cells of undetermined significance) Pap smear results to determine the need for referral to colposcopy. The results of this test are not intended to prevent women from proceeding to colposcopy.
2. In women 30 years and older the HC2 High-Risk HPV DNA Test can be used with Pap to adjunctively screen to assess the presence or absence of high-risk HPV types. This information, together with the physician’s assessment of cytology history, other risk factors, and professional guidelines, may be used to guide patient management.”

TA Criterion 1 is met.

TA Criterion 2: The scientific evidence must permit conclusions concerning the effectiveness of the technology regarding health outcomes.

When evaluating and comparing the utility of various approaches to cervical cancer screening, several things must be kept in mind. Data on test characteristics are usually derived from research settings with optimized testing conditions and are likely to overestimate the results that will be obtained in actual practice. For any test, sensitivity can be improved (and specificity worsened) by lowering the threshold for deciding that a test is positive or by increasing the severity of the cervical histology that is labeled disease. For instance, the sensitivity of conventional Pap cytology for detecting CIN 2 or greater will be increased if the threshold for labeling the test positive is a finding of ASC-US rather than LSIL or HSIL. Similarly, the sensitivity will increase if disease is defined as invasive cancer rather than CIN 2 or higher. For consistency, this review will report the sensitivity of all tests to detect CIN 2 or higher because the goal of Pap testing is to detect and treat pre-malignant as well as malignant disease. In order to maximize the sensitivity of Pap testing in this review, the threshold for calling a Pap test positive will be ASC-US or higher for both conventional and liquid based cytology. A higher threshold (LSIL) would give lower sensitivity, but higher specificity. Similarly, the threshold for a positive HPV test using the HC II method has been set at 1 pg/ml based on extensive evaluation of the tradeoffs between sensitivity and specificity performed by the manufacturer. However, some authors also report their results using a threshold of 2 pg/ml, which decreases the sensitivity of the test in order to increase specificity.

A second major issue in assessing the test characteristics of cervical cancer screening methods is verification bias. In the ideal study, all participants would be tested using the new test and the gold standard. The gold standard for cervical cancer screening is colposcopy with biopsy. Ideally, random four quadrant biopsies would be obtained in addition to biopsy of any suspicious lesions. For ethical and practical reasons, this is rarely done. Of greater concern is the fact that colposcopy is usually not done on women who test negative. Some proportion of women who test negative on the screening test will have cervical dysplasia. Studies with verification bias (not performing the gold standard on patients who test negative) will overestimate the sensitivity of the test. Some studies make statistical corrections for this problem by performing colposcopy on a random sample of women who test negative on the screening test and use these results to extrapolate to the full study population. Unfortunately, the majority of the studies make no attempt to correct for or to eliminate verification bias. Often the most accurate way to evaluate the test characteristics of a new screening test is to compare them to the test characteristics for the standard test (Pap test) done concurrently with the same patients.

There have been several important reviews of the role of HPV testing in cervical cancer screening. Cuzick et al performed a detailed systematic review of the HPV testing in cervical cancer as part of a formal Health Technology Analysis in the United Kingdom in 1999 (Cuzick *et al.* 1999b). The US Preventive Services Task Force reviewed the topic as part of their update on Cervical Cancer Screening completed in January 2003 (USPSTF 2003). Finally, the

American Cancer Society reviewed HPV screening as part of their updated guidelines for cervical cancer screening in November/December 2002 (Saslow *et al.* 2002). These reviews and their bibliographies were carefully screened to identify primary data sources and search strategies to ensure a complete review of the relevant literature.

The literature search for this review identified 24 publications evaluating the test characteristics of HPV testing in screening for cervical cancer. Most studies were cross-sectional or had short-term follow-up. An early study was excluded because the investigators tested for only four of the 13 high-risk HPV types (Cuzick *et al.* 1995). Eight of the studies were early reports or additional analyses using the same patient population as studies included in this review (Belinson *et al.* 1999; Clavel *et al.* 1999; Womack *et al.* 2000a; Womack *et al.* 2000b; Wright *et al.* 2000; Castle *et al.* 2002; Castle *et al.* 2003; Ferreccio *et al.* 2003). Thus, fifteen unique studies assessed the diagnostic performance of HPV testing in over 80,000 women (Cuzick *et al.* 1999a; Kuhn *et al.* 2000; Ratnam *et al.* 2000; Schiffman *et al.* 2000b; Schneider *et al.* 2000; Belinson *et al.* 2001; Blumenthal *et al.* 2001; Clavel *et al.* 2001; Kjaer *et al.* 2002; Kulasingam *et al.* 2002; Coste *et al.* 2003; Cuzick *et al.* 2003; Petry *et al.* 2003; Salmeron *et al.* 2003; Sherman *et al.* 2003). Their methodological characteristics are summarized in Table 2. The majority (13/15) used HC II for HPV testing, though four of the studies initially used an earlier version of the test, Hybrid Capture I (HC I) and only performed HC II testing on a subset of their participants (Cuzick *et al.* 1999b; Kuhn *et al.* 2000; Ratnam *et al.* 2000; Schiffman *et al.* 2000b). Data from 2 studies using PCR alone (Schneider *et al.* 2000; Kjaer *et al.* 2002) and 2 using both PCR and HC II (Cuzick *et al.* 1999a; Kulasingam *et al.* 2002) are included in Table 2 for completeness. The focus of this review will be on the HC II method because the FDA has not approved any PCR-based systems for use in the United States.

Verification bias was avoided or corrected in 54% (7/13) of the studies. The investigators blinded the pathologists' evaluation of Pap tests in all of the studies and their evaluation of biopsy specimens in all but one of the studies. Only 54% of the studies stated that the HPV testing was blinded to Pap test results, but the others did not report either way. Blinded assessment of HPV tests, while ideal, is less important than blinding of cytology and histology readings because the reading is much less subjective.

Several of the early studies were done in high-risk populations, meaning they took place in countries without regular screening programs, and with programs which may not be generalizable to the United States (Kuhn *et al.* 2000; Schiffman *et al.* 2000b; Belinson *et al.* 2001; Blumenthal *et al.* 2001; Salmeron *et al.* 2003). By focusing on studies done in countries with existing screening programs, the test characteristics and screening population are more likely to be comparable to screening in the US.

One recent study has reported on the results of long-term follow-up (Castle *et al.* 2002; Castle *et al.* 2003; Sherman *et al.* 2003) from the most recent follow-up out to ten years. Finally, there is one study that randomized patients with ASC-US on Pap testing or a positive HPV test or both to immediate colposcopy or repeat testing in six months

(Cuzick *et al.* 2003). There are no published studies randomizing participants to one strategy that includes HPV testing and one that does not.

Level of Evidence: 1, 3, 4

Table 2: Methodological characteristics of studies of HPV testing for cervical cancer screening

Reference	N	Age, year (mean)	Patient population	HPV test	Reference Standard	Verification bias avoided	HPV blinded to Pap	Pap blinded to HPV	Histology blinded
Cuzick 1999 London, United Kingdom	2,988	35-70 (46)	Women presenting for routine Pap screening ≥35 years old	HC I 43% HC II 57% PCR MY 09/11 100%	Colposcopy with histology	No	NR	Yes	Yes
Schiffman 2000 Guanacaste, Costa Rica	8,554	18-90 (37)	Women selected randomly door-to-door	HC I 100% HC II 13%	Colposcopy with histology	2% random sample of Pap-HPV-participants referred for colposcopy	NR	Yes	Yes
Ratnam 2000 Newfoundland, Canada	2,098	18-69 (30)	Multiple screening clinics.	HC I 69% HC II 31%	Colposcopy with histology	10% random sample of Pap-HPV-participants referred for colposcopy	NR	NR	NR
Schneider 2000 Jena, Germany	4,761	18-70 (35)	Multiple screening clinics. 8 month follow-up.	PCR GP5+//6+	Colposcopy with histology	By modeling	NR	NR	NR
Kuhn 2000 Cape Town, South Africa	2,861	35-65	Unscreened women. Community based recruitment.	HC I 100% HC II 15%	Colposcopy with histology	No	Yes	Yes	Yes
Belinson 2001 Shanxi, China	1,997	35-45 (39.1)	Unscreened women. Community based recruitment.	HC II	Colposcopy with histology	Yes	NR	Yes	Yes
Blumenthal 2001 Harare, Zimbabwe	2,073	25-55	Primary care clinics. No prior cancer diagnosis.	HC II	Colposcopy with histology	Yes	Yes	Yes	No
Clavel 2001	7,932	15-76 (34)	Normal cytology at enrollment. 15 month follow-up. No	HC II	Colposcopy with histology	No	NR	Yes	Yes

Reference	N	Age, year (mean)	Patient population	HPV test	Reference Standard	Verification bias avoided	HPV blinded to Pap	Pap blinded to HPV	Histology blinded
Reims, France			colposcopy if HPV+ only.						
Kjaer 2002 Copenhagen, Denmark	10,758	20-29 (25)	Population based random sample. Primary focus is longitudinal follow-up.	PCR GP5+/6+	Colposcopy with histology	No	NR	Yes	Yes
Kulasingam 2002 Seattle, Washington	4,075	18-50 (25)	Family planning clinic. Thin prep Pap.	HC II PCR MY09/11	Colposcopy with histology	5% random sample of Pap-HPV-participants referred for colposcopy	NR	Yes	Yes
Sherman 2003 Portland, Oregon	20,786	16-94 (35.9)	Routine screening clinic at HMO.	HC II	Colposcopy with histology	No	Yes	Yes	Yes
Coste 2003 Paris, France	1,757	(33.3)	2 University and 2 private practice clinics.	HC II	Colposcopy with histology	No	Yes	Yes	Yes
Petry 2003 Tubingen & Hanover, Germany	7,908	30-60 (42.7)	Women ≥ 30 years at 2 University clinics.	HC II	Colposcopy with histology	5% random sample of Pap-HPV-participants referred for colposcopy	Yes	Yes	Yes
Salmeron 2003 Morelos, Mexico	7,732	15-85 (42.5)	Patients in a regional cancer screening program.	HC II	Colposcopy with histology	No	Yes	Yes	Yes
Cuzick 2003 United Kingdom	10,358	30-60	Routine screening in primary care clinics.	HC II	Colposcopy with histology	5% random sample of Pap-HPV-participants referred for colposcopy	Yes	Yes	Yes

HPV Human papillomavirus
PPV Positive predictive value
NPV Negative predictive value
LR+ Positive likelihood ratio
LR- Negative likelihood ratio
LSIL Low-grade squamous intraepithelial lesion

HSIL High-grade squamous intraepithelial lesion
CIN Cervical intraepithelial neoplasia
NR Not reported and unable to calculate from data provided in the paper

Pap C Conventional Papanicolaou cytology test
Pap L Liquid-based thin layer Papanicolaou cytology test
HC I Hybrid Capture I test
HC II Hybrid Capture II test
HC II S HC II performed on self collected specimen
HC II C HC II performed on specimen collected using endocervical brush
provided by test manufacturer
PCR Polymerase chain reaction
MY 09/11 PCR using MY 09/11 primers
GP 5+/6+ PCR using GP 5+/6+ primers

Table 3: Performance of screening HPV tests and Pap cytology for the detection of high-grade cervical abnormalities

Reference	N	N disease*	Age, year (mean)	Test	Positive, %	Sensitivity	Specificity	PPV	NPV	LR+	LR-
Cuzick 1999 London, United Kingdom	2,988	42	35-70 (46)	HC II	6.8	95.2	95.1	17.1	99.9	19	0.05
				HC I	19.9	70.4	81.7	4.4	99.5	3.8	0.36
				PCR	5.9	73.8	96.6	17.5	99.6	22	0.27
				Pap C	5.6	85.7	97.5	21.7	99.8	34	0.15
Schiffman 2000 Guanacaste, Costa Rica	8,554	138	18-90 (37)	HC II	12.3	88.4	89.0	-	-	8.0	0.13
				HC I	7.7	74.8	93.4			11	0.27
				Pap C	6.9	77.7	94.2			13	0.24
Ratnam 2000 Newfoundland, Canada	2,098	30	18-69 (30)	HC I/II	10.8	68.1	90.6	15.4	99.1	7.2	0.35
				Pap C	9.2	40.2	91.6	10.7	98.4	4.8	0.65
Schneider 2000 Jena, Germany	4,761	114	18-70 (35)	PCR	7.8	89.4	93.9	35.8	99.6	15	0.11
				Pap C	0.9	20.0	99.2	70.6	97.5	25	0.81
Kuhn 2000 Cape Town, South Africa	2,861	86	35-65	HC II	22.0	88.4	81.9	19.0	-	4.9	0.14
				HC I	16.2	73.3	87.8	23.5		6.0	0.30
				Pap C	15.2	78.3	96.8	31.9		24	0.22
Belinson 2001 Shanxi, China	1,997	86	35-45 (39.1)	HC II C	18	95.3	85	22.5	99.8	6.4	0.06
				HC II S	17	82.6	86	21.9	99.1	5.9	0.20
				Pap L	25	94.2	78	16.0	99.7	4.3	0.07
Blumenthal 2001 Harare, Zimbabwe	2,073	208	25-55	HC II	44	80.1	61.1	18.1	96.5	2.1	0.33
				Pap C	12	44.3	90.6	33.3	93.6	4.7	0.61
Clavel 2001 Reims, France	7,932	129	15-76 (34)	HC II	15.3	100	86.1	10.6	100	7.2	0.00
				Pap L	9.1	87.8	93.1	15.7	99.8	12.7	0.13
				Pap C	6.0	68.1	95.3	23.5	99.3	14.5	0.33
Kjaer 2002 Coopenhagen,	10,758	165	20-29 (25)	PCR	NR	93%	NR	NR	NR	NR	NR

Reference	N	N disease*	Age, year (mean)	Test	Positive, %	Sensitivity	Specificity	PPV	NPV	LR+	LR-
Denmark											
Kulasingam 2002	4,075	CIN 3+ 87	18-50 (25)	HC II	28.4	90.8	72.6	6.7	99.6	3.3	0.13
				PCR	18.3	88.2	78.8	8.3	99.5	4.2	0.15
Seattle, Washington				Pap L	16.6	61.3	82.4	7.1	98.5	3.5	0.47
	760	CIN 2+ 137		HC II	NR	62.7	83.0	11	98.5	3.7	0.45
		Age≥30		PCR	NR	56.5	87.3	13	98.3	4.4	0.50
				Pap L	NR	38.3	86.4	8.7	97.6	2.8	0.71
Sherman 2003	20,786	CIN 3+ 118	16-94 (35.9)	HC II	14.3	75.4	86.0	3.0	99.8	5.4	0.29
		Within 45 months		Pap C	3.1	49.1	97.1	8.9	99.8	17	0.52
Portland, Oregon											
Coste 2003	1,757	41	(33.3)	HC II	17.0	96	85	13	99.9	6.4	0.05
				Pap L	13.4	87.5	88.3	14.9	99.7	7.5	0.14
Paris, France				Pap C	12.4	87.8	89.4	16.6	99.7	8.3	0.14
Petry 2003	7,908	86	30-60 (42.7)	HC II	5.2	97.8	95.3	10.9	100	21	0.02
				Pap C	2.2	43.5	98.0	11.4	99.7	22	0.58
Tubingen & Hanover, Germany											
Salmeron 2003	7,732	101	15-85 (42.5)	HC II C	9.3	93.1	91.8	14.9	100	11.4	0.08
				HC II S	11.6	71.3	89.2	9.1	99.7	6.6	0.32
Morelos, Mexico				Pap C	2.4	59.4	98.3	36.1	99.5	34.9	0.41
Cuzick 2003	10,358	90	30-60	HC II	7.6	97.1	93.3	12.8	99.9	14.5	0.03
				Pap C	4.8	76.6	95.8	15.8	99.6	18.2	0.24
United Kingdom											

* Disease is "CIN 2+" = CIN 2, CIN 3 or cancer unless otherwise noted.

TA Criterion 3: The technology must improve the net health outcomes.

The potential benefits of HPV testing as a screening test are not documented in prospective cohort studies or trials that evaluate outcomes among women who received conventional cytology alone compared to an alternate strategy that includes HPV testing. HPV testing is intended to increase the sensitivity of cervical cancer screening programs and thus has the potential to decrease cervical cancer mortality through earlier detection and treatment and to decrease cervical cancer incidence through the detection and treatment of high-grade dysplasia. Furthermore, women with negative Pap smears and negative HPV tests may be able to increase the interval between screening tests.

No studies were identified which explicitly addressed potential harms of HPV testing. Concerns that have been raised in the literature include increased anxiety and decreased quality of life among women labeled as carrying a sexually transmitted disease that can cause cancer, particularly given the lack of an effective treatment for the infection. Increased anxiety has the potential to decrease follow-up with patients or conversely, to lead to over-treatment. HPV testing could undermine the importance of cytologic screening resulting in decreasing rates of screening in the population and a reversal in the declining incidence of cervical cancer in the population. The diagnosis of HPV infection may also cause conflict between partners. Finally, there are economic costs associated with increased surveillance and additional colposcopies.

There are no published studies directly assessing the impact of HPV testing on cervical cancer outcomes. However, there are at least 15 studies that assess the sensitivity and specificity of HPV testing compared to Pap testing. One of these studies has long-term follow-up that allows for accurate estimates of the risk of developing cervical cancer based on HPV test results. There is also one high quality randomized clinical trial assessing different strategies for managing patients who have normal Pap cytology, but test positive for oncogenic HPV. Together, these data permit assessment of the net benefit of HPV testing, although data on potential harms is limited.

The test characteristics of HPV testing and Pap testing performed concurrently are summarized in Table 3. HPV testing is consistently more sensitive than Pap cytology in these 15 studies. The average absolute improvement in sensitivity for HC II compared with the conventional Pap test is about 26% (range 8-36% with two outliers: 1% and 54%). However, absolute specificity is decreased by an average of about 7.6% (range 1-14.9%, one outlier 29.5%). Dropping the outliers or limiting the analyses to studies done in developed nations with established cervical cancer screening programs had no significant impact on these estimates. Limiting HPV screening to women 30 years and older would increase the specificity as women in this age group are less likely to have new transient HPV infections. Studies of women in this age group in countries with established screening programs (Cuzick *et al.* 1999a; Kulasingam *et al.* 2002; Cuzick *et al.* 2003; Petry *et al.* 2003) had an average improvement in sensitivity of 31% with a decrease in specificity of only 2.6%.

The study by Kulasingam et al (2002) was the first publication on the test characteristics of HPV testing in a US screening population. Their goal was to assess the accuracy of HPV DNA testing for detecting CIN 3 or cancer. Between December 1997 and October 2000, 4075 women who attended Planned Parenthood clinics in Washington State were screened simultaneously using liquid based monolayer Pap and HPV DNA testing by a PCR and by HC II. Women who were positive for high-risk HPV types, or who had Pap results of ASCUS or higher, were considered to have positive screening test results and were referred for colposcopy and biopsy. Additionally, a 5% random sample of women with negative screening test results was referred for colposcopy to permit statistical correction for verification bias. The estimated prevalence of CIN 3 or higher was 3.2%. The sensitivity (95% confidence interval) of thin-layer Pap (with a result \geq ASC-US) for identifying women with CIN 3 or higher was only 61.3% (48.5%-70.9%) compared with 88.2% (78.9%-93.8%) for HPV testing by PCR and 90.8% (83.1%-95.8%) by HC II. Differences in specificities were also observed: 82.4% (81.8%-83.1%) for thin-layer Pap (with a result \geq ASC-US), 78.8% (77.9%-79.7%) for PCR, and 72.6% (69.4%-75.0%) for signal amplification. Compared with referral for colposcopy of all women with ASCUS or higher, HC II testing of women with ASCUS and referral of those with a positive result was about as sensitive (61.3% vs 60.3%, respectively) and significantly more specific (82.4% vs. 88.9%, respectively). The strategy requiring repeat positive PCR tests on two visits had a sensitivity of 84.2% (75.3%-91.0%) and a specificity of 86.2% (85.1%-87.3%). All tests were more specific and less sensitive in older \geq 30 years) vs. younger women. Because CIN 2 is considered high grade dysplasia and is usually treated, test characteristics for detecting CIN 2 or higher were also reported. In women 30 years and older, thin-layer Pap for CIN 2 had a sensitivity of 38% and a specificity of 86%. The HC II test in the same population had a sensitivity of 63% and a specificity of 83%. The authors conclude that testing for HPV has higher sensitivity but lower specificity than thin-layer Pap screening and argue that in settings with long or haphazard screening intervals, screening for HPV DNA may be a reasonable alternative to cytology-based screening.

A second, large US study by Sherman et al (2003) evaluated whether simultaneous screening with a Pap test and HPV testing is useful for assessing the risk for CIN 3 or cervical cancer. They enrolled 23,702 subjects in a study of HPV infection at Kaiser Permanente, Portland, OR. Data were analyzed for 20,810 volunteers who were at least 16 years old (mean = 35.9 years) with satisfactory baseline Pap tests and suitable samples for HPV testing. Women were followed for up to 122 months (from April 1, 1989, to June 30, 1999) to determine the risk for histopathologically confirmed CIN 3 or cancer. The long follow-up period was used to correct for potential verification bias. Among 171 women with CIN 3 or cancer diagnosed over 122 months, 123 (71.9%, 95% confidence interval [CI] = 65.2% to 78.7%) had baseline Pap results of atypical squamous cells or worse and/or a positive HPV test, including 102 (86.4%, 95% CI = 80.3% to 92.6%) of the 118 cases diagnosed within the first 45 months of follow-up. During this 45-month period, the cumulative incidence of CIN 3 or cancer was 4.54% (95% CI = 3.61% to 5.46%) among women with a Pap

test result of atypical squamous cells or worse, positive HPV tests, or both compared with 0.16% (95% CI = 0.08% to 0.24%) among women with negative Pap and HPV tests. The authors concluded that normal baseline Pap and HPV tests were associated with a low risk for CIN3 or cancer in the subsequent 45 months, largely because a negative HPV test was associated with a decreased risk of cervical neoplasia. Negative combined test results should provide added reassurance for lengthening the screening interval among low-risk women, whereas positive results identify a small subgroup that requires more frequent surveillance.

A more recent study (Cuzick *et al.* 2003) in the United Kingdom addressed the issue of how to manage HPV-positive women with negative or borderline cytology. They compared the detection rate and positive predictive values of HPV assay with cytology to determine the best management strategy for HPV-positive women in a multicenter screening study of 11,085 women aged 30-60 years. Women with borderline cytology and women positive for high-risk HPV with negative cytology were randomized to immediate colposcopy or to surveillance by repeat HPV testing, cytology, and colposcopy at 12 months. HPV testing was more sensitive than borderline or worse cytology (97.1% vs. 76.6%, $p=0.002$) but less specific (93.3% vs. 95.8%, $p<0.0001$) for detecting CIN 2+. Of 825 randomized women, surveillance at 12 months was as effective as immediate colposcopy. In women positive for HPV at baseline, who had surveillance, 73 (45%) of 164 women with negative cytology and eight (35%) of 23 women with borderline cytology were HPV negative at 6-12 months. No CIN 2+ was found in these women, nor in women with an initial negative HPV test with borderline ($n=211$) or mild (32) cytology. The authors suggest that HPV testing could be used for primary screening in women older than 30 years, with cytology used to triage HPV-positive women. HPV-positive women with normal or borderline cytology (about 6% of screened women) could be managed by repeat testing after 12 months. This approach could potentially improve detection rates of CIN 2+ without increasing the colposcopy referral rate.

The literature clearly demonstrates that HPV testing is more sensitive than Pap cytology in screening populations around the world. Thus, if used alone for screening, it should improve net health outcomes compared to no screening. The combination of the two tests is even more sensitive, though with lower specificity. Improved sensitivity alone is not sufficient to recommend widespread HPV testing because it is not known how often to screen women who test negative for HPV, nor how to manage women with normal Pap cytology who test positive for oncogenic HPV types. The prospective cohort study of Sherman *et al.* (2003) found that the incidence of cervical neoplasia over 45 months is very low (0.16%) in women with normal Pap cytology and negative HPV test results. This provides evidence that a screening interval of at least 3 years for women testing negative on both tests is reasonably safe. Finally, the randomized clinical trial of Cuzick *et al.* (2003) found that repeat screening after one year in women with normal Pap cytology and a positive HPV test is as safe as immediate colposcopy. Together, these results suggest a reasonable approach for adding HPV testing to cervical cancer screening programs. None of these studies directly

compare screening with HPV testing versus screening without HPV testing. Indeed, Cuzick et al. suggest that the correct approach to screening is to start with HPV testing alone, because it is the more sensitive test. They call for a large clinical trial comparing screening with HPV testing alone, to a strategy based on Pap smears.

TA Criterion 3 is met.

TA Criterion 4: The technology must be as beneficial as any established alternatives.

The current standard for cervical cancer screening is annual Pap testing. There are no clinical trials with randomized, concurrent, or historical controls directly comparing any screening strategy incorporating HPV testing to standard Pap testing. Given the lack of clinical trial evidence, we must turn to modeling to assess the overall effectiveness and cost-effectiveness of HPV screening. As part of a comprehensive review of HPV testing done in the UK, Cuzick et al compared combined HPV and Pap testing, replacing Pap testing with HPV testing, and adding HPV testing for surveillance of low-grade lesions versus conventional Pap testing (Cuzick *et al.* 1999b). The authors concluded “The uncertainty, as expressed by the differences between models, is so large, the results are inconclusive. Adding HPV testing to cervical cancer screening may or may not improve the effectiveness of screening.”

As can be seen from Tables 2 and 3, there is an explosion of new data on HPV testing and on cervical cancer screening in general. A more recent cost-effectiveness model compared the societal costs and benefits of HPV testing, Pap testing, and their combination to screen for cervical cancer (Mandelblatt *et al.* 2002). A simulation model of the natural history of cervical dysplasia was used to estimate the societal costs and quality-adjusted life expectancy associated with 18 different general population screening strategies, including Pap plus HPV testing, Pap testing alone, and HPV testing alone every two or three years among hypothetical longitudinal cohorts of US women. Maximal savings in lives were achieved by screening every two years until death with combined HPV and Pap testing at an incremental cost of \$76,183 per quality adjusted life year (QALY) compared with Pap testing alone every two years. Stopping biennial screening with HPV and Pap testing at age 75 years captures 97.8% of the benefits of lifetime screening at a cost of \$70,347 per QALY. Human papillomavirus screening alone was equally effective as Pap testing alone at any given screening interval or age of screening cessation, but was more costly and therefore was dominated. In sensitivity analyses, HPV testing would be more effective and less costly than Pap testing at a cost threshold of \$5 for an HPV test. The authors argue that screening with HPV plus Pap tests every two years appears to save additional years of life at reasonable costs compared with Pap testing alone. However, expenses higher than \$50,000 per QALY are usually not considered cost effective. The model does not allow for the current practice of differential screening intervals based on prior test results nor does it adequately adjust for the changes in the natural history of HPV infection and test characteristics with aging. More detailed models including data published in the last two years are needed.

Recently, guidelines from the ACS (also adopted by ACOG) were adopted which support HPV testing for women over age 30. They recommend that women with normal Pap tests who test negative for HPV should be re-screened no more frequently than every three years. However, they specifically label this as a “preliminary recommendation” and call for guidelines to be developed for management of women with normal Pap smears who test positive for HPV DNA. They specifically note that “the prognostic value of a positive test result, especially in the absence of a cytologic abnormality, has not been fully validated in prospective studies.” (Saslow *et al.* 2002). The recent randomized clinical trial by Cuzick et al (Cuzick *et al.* 2003) suggests that they may be safely managed by repeat testing in twelve months with colposcopy reserved for women who have persistent HPV infection or abnormalities at six months.

The incorporation of HPV DNA testing into primary screening for cervical cancer will result in millions of women with normal Pap tests being told that they are at increased risk for cervical cancer. It is important that strategies be put in place to make sure that these women are neither over-treated nor unduly alarmed or stigmatized by their diagnosis. In the absence of studies that related HPV testing to health outcomes, the linkages between comparative test performance of HPV testing and conventional cytology are insufficient to judge the implications of using one strategy over another.

Furthermore, there are a number of ongoing, large randomized clinical trials comparing screening strategies for cervical cancer that include HPV testing to alternate strategies. In England, the ARTISTIC study randomized 28,000 women ages 20-64 years to receive either liquid-based monolayer Pap testing plus HPV testing or Pap testing alone and is following the women for six years. Five counties in Sweden are participating in SWEDESCAN which randomized 10,000 women to either Pap testing and HPV testing or Pap testing alone. In the Osmanabad Randomized Clinical Trial, approximately 120,000 previously unscreened women will be randomized to four arms: HPV testing, Pap testing, visual inspection of the cervix with acetic acid or control and followed for incident cervical cancer. The Canadian trial CCaST is randomizing 28,000 women ages 30-69 years to HPV testing followed by Pap testing or Pap testing followed by HPV testing. All women with ASC-US or HPV will receive colposcopy and if the colposcopy is negative, it will be repeated in 6 months. A random sample of women with normal Pap cytology and a negative HPV test will also receive colposcopy. There is also a large, ongoing HPV testing demonstration project in the Netherlands that should have results soon. The most relevant studies weighing the relative risks and benefits of HPV testing are ARTISTIC and SWEDESCAN as they are being done in populations with cervical screening practices similar to the United States and they are directly comparing strategies with and without HPV testing. The Canadian CCaST study should provide more precise estimates for the sensitivity of HPV testing with or without Pap testing that are adjusted for verification bias.

When comparing HPV testing to the current standard, Pap testing, the key group to focus on is women with normal Pap cytology who test positive for HPV. They represent the group that may benefit, as they are identified as higher risk than they would have been without testing. Without knowing the HPV test results, it is usually recommended that women with normal Pap test results have repeat Pap testing in one year. Given the results of the HART study (Cuzick, 2003), women with normal cytology who test positive for oncogenic HPV types would also be counseled to have repeat testing in one year. If they remain positive for HPV or have abnormal cytology, colposcopy would be recommended. The primary difference in referrals between the two groups would be for women who continue to have normal cytology on Pap testing at one year and have a persistent HPV infection. If the Pap cytology is abnormal, both groups would be treated similarly. Women with normal cytology who test positive for oncogenic HPV are also the women most likely to be harmed by the test. They will be told that they are infected with a sexually transmitted virus that can cause cancer. This will undoubtedly increase anxiety significantly for some women, which may lead to poor quality of life, demands for immediate and often unnecessary colposcopy, or avoidance of future cervical cancer screening. On the other hand, the increased knowledge about their risk for cervical cancer could lead to improved compliance with screening recommendations. Given the lack of comparative data, it is not clear that the addition of HPV testing improves cervical cancer screening. The ongoing randomized clinical trials should help resolve these issues.

TA Criterion 4 is not met.

TA Criterion 5: The improvement must be attainable outside the investigational settings.

Specimen collection for HPV testing with the HC II system is similar to that required for Pap testing and is certainly feasible in routine practice. Similarly, the technical steps required to process and test the specimen are standard in any modern medical laboratory facility. However, improvements in health outcomes have not yet been demonstrated in the investigational setting.

TA Criterion 5 is not met.

OPINIONS OF OTHERS

Blue Cross Blue Shield Association (BCBSA)

The Blue Cross Blue Shield Association Technology Evaluation Center Medical Advisory Panel has not reviewed this topic.

Centers for Medicaid and Medicare Services (CMS)

In November 2001, the Centers for Medicaid and Medicare Services issued a Program Memorandum to its intermediaries and carriers directing them to promote cervical cancer screening through education and the use of Pap tests. The CMS position specific to the use of HPV testing was not available.

U.S. Preventive Services Task Force (USPSTF)

In January 2003 the USPSTF issued Recommendations and Rationale for screening for Cervical Cancer and noted that “The USPSTF concludes that the evidence is insufficient to recommend for or against the routine use of human papillomavirus (HPV) testing as a primary screening test for cervical cancer. The USPSTF found poor evidence to determine the benefits and potential harms of HPV screening as an adjunct or alternative to regular Pap smear screening. Trials are underway that should soon clarify the role of HPV testing in cervical cancer screening.”

American Cancer Society (ACS)

On October 21, 2003 the American Cancer Society revised the Detailed Guide: Cervical Cancer. The following is noted: “The American Cancer Society recommends the following guidelines for early detection:

- Another reasonable option for women over 30 is to get screened every 3 years (but not more frequently) with either the conventional or liquid-based Pap test, *plus* the HPV DNA test.”

American College of Obstetrics and Gynecology (ACOG)

ACOG Practice Bulletin Number 45, August 2003 notes the following:

“The following recommendations are based on limited and inconsistent scientific evidence (Level B):

- The use of a combination of cervical cytology and HPV DNA screening is appropriate for women aged 30 years and older. If this combination is used, women who receive negative results on both tests should be rescreened no more frequently than every 3 years.”

The California chapter of ACOG has been invited to send a representative to participate in the meeting.

Association of Northern California Oncologists (ANCO)

ANCO did not provide representation at the meeting but did provide opinion in favor of the use of HPV testing.

Medical Oncology Association of Southern California (MOASC)

MOASC was not able to provide representation or an opinion at the meeting.

California Society of Pathologists

CSP provided representation at the meeting who provided opinion in favor of the use of HPV testing.

CONCLUSION

HPV testing is a promising approach to cervical cancer screening. Over the past two decades it has become clear that infection with certain high-risk types of HPV is necessary for the development of most cervical cancer. The current generation of tests for HPV infection can accurately detect who is infected with the virus. Fortunately, prospective cohort studies suggest that most women (over 90%) who are infected with HPV clear the infection without developing high-grade cervical dysplasia or invasive cancer. However, this is potentially a large problem with the specificity and positive predictive value of HPV testing for the detection of dysplasia or cancer. Thus HPV testing is not recommended for young, sexually active women who may acquire and clear multiple HPV infections. Most guidelines that advocate the use of HPV testing recommend that it be used only for women 30 year and older.

Few of the published studies limited their analysis to women over the age of 30. Those that do report that the absolute sensitivity of HPV testing with HC II is about 30% greater than Pap testing for the detection of CIN 2 or higher with only a 3% decrease in absolute specificity. Studies that included younger women, often in populations not previously screened for cervical cancer report somewhat smaller gains in sensitivity and significantly larger decreases in specificity. The trade off between increased sensitivity with decreased specificity has the potential to significantly increase the number of women sent for further evaluation with colposcopy. If both Pap and HPV testing are performed, there will be an even greater number of false positives.

Because few of these studies evaluate outcomes among women whose subsequent management was based on HPV testing and because only one is experimental, we cannot draw direct conclusions about the net health outcomes of cervical cancer screening programs incorporating HPV testing. Some authors have suggested that the most rationale screening approach would start with HPV testing, as HPV testing is significantly more sensitive than cervical cytology. Pap smears would only be performed on patients who test positive for oncogenic HPV strains. Another rationale strategy would be to only perform HPV testing for women whose Pap cytology is normal or ASC-US. Current American Cancer Society guidelines labeled as “preliminary” suggest that it is acceptable to do both Pap testing and HPV testing concurrently in women over 30 provided that women who test negative are screened no more frequently than every three years. However, no guidance is provided for the management of women who have normal Pap tests, but are HPV positive. The balance of benefits and harms to women in this group is crucial in determining whether the addition of HPV testing has any advantages over the current standard: Pap testing. HART, the randomized clinical trial by Cuzick et al, suggests that a reasonable approach would be to repeat the Pap test and HPV test in 12 months and only perform colposcopy on women who have persistent abnormalities (Cuzick *et al.* 2003). However, no published studies have demonstrated that the additional resources needed to implement HPV testing leads to a decrease in incident cervical cancer or mortality.

Thus, many unanswered questions remain about the optimal strategy for incorporating HPV testing into cervical cancer screening programs. Alternative strategies that have been proposed include using HPV as the primary test and performing Pap testing only on patients testing positive for HPV, starting with Pap testing and performing HPV testing only on patients with normal or ASC-US on cytology, or performing both tests concurrently. Current models suggest that Pap and HPV testing every two years would maximize life expectancy, but the approach was not cost-effective by current standards. Furthermore, there are limited data on the best way to manage patients who test positive for HPV, but have normal cervical cytology test. Most women will clear the infection within one to two years, but there are no treatments, no way to predict who will clear the infection, and no data on the impact that a positive “high-risk for cancer” HPV test will have on a woman’s quality of life. It is also not clear what screening interval is appropriate for women who test negative for HPV and have a normal Pap. Current guidelines suggest no more frequently than every three years, but the optimal screening interval has not been determined.

Current evidence suggests that a reasonable way to incorporate HPV testing into current cervical screening programs would be as follows:

1. HPV testing is not recommended for women under the age of 30
2. HPV testing may be offered to women 30 years and older in conjunction with Pap testing
 - Women who test negative on both should not be screened again for at least 3 years
 - Women who test positive for HPV, but have normal Pap cytology should have repeat screening tests in 6-12 months
 - Women with abnormal Pap cytology should be treated according to current guidelines

Understanding the best way to add HPV testing to the current screening approach will depend on incorporating the results of ongoing randomized trials into current models of HPV screening. Until these data are available, the evidence is insufficient to recommend for or against the incorporation of HPV testing into cervical cancer screening programs. Finally, it is important to recognize that the majority of the cases of cervical cancer that are diagnosed in the United States are in women who have never received any screening for cervical cancer or have not been screened for over five years. Thus, the potential public health impact of screening with HPV testing will remain small unless there are increases in the proportion of women at risk for cervical cancer who are tested.

RECOMMENDATION

It is recommended that the use of Human Papillomavirus Testing in Cervical Cancer Screening does not meet technology assessment criteria 4 or 5 for safety, effectiveness, and improvement in health outcomes.

The California Technology Assessment Forum approved the recommendation as presented.

February 11, 2004

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