REVIEW

CME CREDIT

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New cervical cancer screening strategy: Combined Pap and HPV testing

ABSTRACT

Our strategy for cervical cancer screeing is being revolutionized by our new understanding of how human papillomavirus (HPV) contributes to carcinogenesis and the natural history of cervical cancer. The American Cancer Society and the American College of Obstetricians and Gynecologists now recommend combined HPV and Papanicolaou (Pap) testing for cervical cancer screening in women age 30 or older. However, although incorporation of HPV DNA testing into primary screening provides clear benefits, it also raises new questions.

KEY POINTS

HPV infection most often is transient in younger women. With increasing age, the likelihood increases that HPV positivity represents persistent disease, and only those who have persistent high-risk HPV infection are at risk of cervical cancer.

Combined HPV DNA testing and Pap testing is now recommended for primary screening in women age 30 or older. If both tests are negative, the screening interval can be extended to every 3 years.

If a woman has a positive result on HPV testing but a negative result on Pap testing, she should repeat both tests in 6 to 12 months.

Eventually, the search for ideal cervical cancer biomarkers will improve risk stratification in screening, while an HPV vaccine will eradicate cervical cancer.

WOMEN AGE 30 and older may undergo combined Papanicolaou (Pap) and human papillomavirus (HPV) testing to screen for cervical cancer, according to new guidelines from several professional societies.[1–3] If both test results are negative, subsequent screening can be at 3-year intervals.

These recommendations came after the US Food and Drug Administration (FDA) approved the HPV test (Hybrid Capture 2; Digene Corporation, Gaithersburg, MD) as an adjunct for primary cervical cytology screening. The United States Preventive Services Task Force (USPSTF),[4] however, finds that there is insufficient evidence to recommend for or against its routine use for this purpose.

Up to now, the HPV test has been recommended and approved only as a follow-up test for women with a Pap test finding of atypical squamous cells of undetermined significance (ASC-US).[5] For women younger than 30 years, screening is still every year with conventional Pap testing or every 2 years with ThinPrep Pap testing (or every 3 years according to the USPSTF).

These are exciting times in the field of cervical cancer detection and prevention, as progress in understanding the role of HPV in carcinogenesis is being applied to clinical practice (**TABLE 1**).[6–13]

This article briefly reviews contemporary concepts of cervical cancer carcinogenesis, evidence supporting HPV testing in primary screening, current practice guidelines, commonly asked questions, and future directions in screening.

ROLE OF HPV IN CERVICAL CANCER

HPV infection is necessary for cervical cancer to develop but does not suffice by itself.[14–18]

TABLE 1

Recent milestones in cervical cancer screening

1996	The FDA approves liquid-based ThinPrep technology, significantly increasing rates of specimen adequacy and cytologic diagnosis of cervical cancer precursors and decreasing ambiguous interpretations[6–8]
2000	The FDA approves HPV DNA test for testing women with an abnormal Pap test to determine if they need colposcopy
2001	The Bethesda terminology for Pap smear reporting is revised, reducing ambiguity and allowing better clinical decisions[9,10]
2001	The Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesions (ASCUS/LSIL) Triage Study (ALTS trial) validates the clinical effectiveness of HPV testing in women with mildly abnormal cervical cytologic findings[11–13]
2003	The FDA approves the Hybrid Capture 2 HPV DNA test for women of all ages with ASCUS and for women age 30 or older in routine primary screening

To date, more than 80 HPV types have been identified, and more than 30 of these can infect the genital tract.[19] Certain genital HPV types (16 and 18) are associated with a substantially higher risk of cervical cancer than other types. HPV types that carry a moderate risk of cervical cancer include 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82.[20] Types 6 and 11 carry a low risk.

HPV infection precedes the development of cytologic abnormalities

Extensive studies provide compelling evidence that infection of the cervix with one of the 15 high-risk or moderate-risk HPV types is required for the development of virtually all cervical cancers.[21]

A multicenter study from 22 countries found that HPV DNA could be detected in 93% of squamous cell carcinomas of the cervix.[15] Furthermore, HPV DNA can also be isolated from metastatic cervical cancer tissues and from cervical cancer tumor cell lines in vitro.[22,23]

Finally, in vitro studies are shedding light on the mechanism by which HPV infection increases the risk of cancer (**Mechanisms of HPV oncogenesis** on page 144).[24–33]

WHY HPV-PLUS-PAP TESTING IS THE NEW STANDARD OF CARE

Combining the HPV test with the Pap test in primary cervical cancer screening is the logical extension of the knowledge acquired over the past 2 decades on the natural history of HPV infection and cervical cancer development.

Pap testing lacks sensitivity

For the last 5 decades, annual Pap testing has been the standard of care in screening for cervical cancer. It has decreased both the incidence of cervical cancer and the number of deaths due to cervical cancer by about 75%.[34]

However, in routine screening, the estimated true sensitivity of the conventional Pap test is only 50% to 60%.[35–37] Pap screening is successful, despite this relative insensitivity, because patients undergo repeated testing.

The new liquid-based ThinPrep technology (Cytyc Corporation, Boxborough, MA) has improved the sensitivity of Pap testing. Yet Pap testing may still miss 15% to 35% of cases of cervical intraepithelial neoplasia grade 3 (CIN 3, a precursor of cancer) or cancer itself.[38]

In addition, Pap tests must be interpreted by a pathologist, and results are not very reproducible. And pathologists who, despite their best efforts, failed to detect CIN or cervical cancer on conventional Pap smears have been exposed to increasing numbers of lawsuits.[39–41] Therefore, the conventional Pap smear by itself no longer meets the expectations of clinicians and patients.

HPV testing is more sensitive

In a search for a more sensitive screening test, multiple large-scale studies from many countries evaluated the role of HPV testing in primary screening (**TABLE 2**).[38,42–45] Important findings from these studies:

TABLE 2

Combined HPV and Pap testing in primary screening

STUDY	LOCATION	NO. OF WOMEN	SENSITIVITY (%)*			SPECIFICITY (%)*			NEGATIVE PREDICTIVE VALUE*
			PAP	HPV	PAP+HPV	PAP	HPV	PAP+HPV	
Petry et al[45]	Germany	7,592	34	86	94	99	97	96	0.999
Cuzick et al[1]	United Kingdom	10,358	72	97	100	99	94	93	1.000
Salmeron et al[42]	Mexico	6,115	57	94	98	99	94	94	1.000
Schiffman et al[1]	Costa Rica	6,176	80	86	92	95	94	90	0.998
Belinson et al[43]	China	1,936	94	98	100	78	85	70	1.000
Womack et al[1]	United States	1,040	60	100	100	98	97	96	1.000

*For CIN 2+ = cervical intraepithelial neoplasia grades 2 and 3 or cancer

BASED ON WRIGHT TC JR, SCHIFFMAN M, SOLOMON D, ET AL. INTERIM GUIDANCE FOR THE USE OF HUMAN PAPILLOMAVIRUS DNA TESTING AS AN ADJUNCT TO CERVICAL CYTOLOGY FOR SCREENING. OBSTET GYNECOL 2004; 103:304–309.

- The high-risk HPV DNA test was positive in 80% to 100% of cases of histologically confirmed CIN 2 or cancer.
- HPV testing was more sensitive in detecting CIN 2, CIN 3, or cancer than a single Pap test. (It was, however, less specific. For this reason, HPV testing cannot replace Pap testing. Combined, the two tests have a specificity of 70% to 96%.)
- When HPV testing was combined with a Pap smear, the sensitivity was even higher than that of HPV testing used alone.
- Most important: the combination of a negative Pap smear and a negative HPV test indicated absence of CIN 3 or cancer to a certainty of almost 100%.

Specificity of HPV testing increases with age

Women who test positive for HPV on more than one occasion do not necessarily have persistent infection with the same type of high-risk HPV, nor will they necessarily go on to develop cervical cancer.

Sherman et al[46] reported that the prevalence of high-risk HPV infection declines with age: only 31.2% among women with ASCUS who were 29 years or older, compared with 65% in those age 28 and younger. HPV infection most often is transient in younger women. With increasing age, the likelihood increases that HPV positivity represents persistent disease, and only those who have persistent high-risk HPV infection are at risk of cervical cancer.

As a result, both the specificity and the positive predictive value of an HPV test increase with the age of the patient. Therefore, combined HPV-plus-Pap testing in women age 30 years or older is the new standard of care in cervical cancer screening.

A negative Pap-plus-HPV test nearly rules out CIN 3 or cancer

POTENTIAL HARM FROM HPV TESTING

Adding HPV testing to Pap testing brings clear potential benefits but also poses the risks of overuse and unnecessary invasive treatment.

HPV infection is very common in women, but few of these women will develop cervical cancer or a high-grade precancerous lesion. Combined HPV-plus-Pap testing will identify 10% to 20% of adult women as having transient, clinically insignificant HPV infection.

It is very important to restrict HPV testing to women age 30 or older, to provide adequate counseling regarding their risk of cervical cancer, and to avoid unnecessary invasive therapy such as the loop electrosurgical excision procedure (LEEP).[1]

CURRENT GUIDELINES FOR SCREENING

In view of recent advances (TABLE 1), the American Cancer Society, the USPSTF, and the American College of Obstetricians and

Mechanisms of HPV oncogenesis

Evidence about the mechanism by which HPV contributes to oncogenesis comes from in vitro studies, in which human epithelial cells that are infected with high-risk types of HPV become immortal.[24,25] Other in vitro studies have identified two HPV viral gene products, the proteins E6 and E7, that are necessary for immortalization.[26–28]

E6 proteins from high-risk HPV types interact with the cellular tumor-suppressor protein p53. In noninfected cells, p53 levels increase in response to cellular or DNA damage or aberrant cell proliferation signals. High levels of p53 cause the cell to stop growing in the G1 phase of the cell cycle and allow it to either repair damaged DNA before the next round of DNA synthesis or be eliminated through programmed cell death (apoptosis).[29]

In HPV-infected cells, the E6 protein binds to p53, resulting in rapid proteolytic degradation of the bound p53 through a ubiquitin-dependent pathway.[30,31] The decreased level of p53 diminishes the cell's ability to control the cell cycle and repair DNA damage and ultimately leads to uncontrolled cell growth.[31]

In contrast, E6 proteins from low-risk HPV types do not bind p53 in detectable levels and have no effect on p53 stability in vitro. This weak affinity for p53 may explain the lesser oncogenic potential of the low-risk HPV types.

Similarly, E7 proteins from high-risk HPV types interact with another cellular tumor-suppressor protein, the retinoblastoma protein (pRB). The binding of E7 proteins to pRB disrupts the complex between the cellular transcription factor E2F-1 and pRB. This results in the release of E2F-1, allowing it to stimulate cellular DNA synthesis and uncontrolled cell growth.[32] Again, the E7 protein from low-risk HPV types 6 and 11 binds pRB with a much weaker affinity.

A recent study also suggests a model whereby HPV-16 E7 protein induces centrosome-related mitotic disturbances that are potentiated by HPV-16 E6 protein.[33]

Gynecologists have developed practice guidelines (TABLE 3).[1–4]

The American Cancer Society and American College of Obstetricians and Gynecologists both recommend adding HPV testing to Pap testing in women 30 years and older in primary screening.

To help provide guidance for physicians when using HPV testing as an adjunct to Pap testing for screening, the National Institutes of Health National Cancer Institute, the American Society of Colposcopy and Cervical Pathology, and the American Cancer Society cosponsored a workshop in 2003. It is the consensus from the workshop that HPV testing may be added to the Pap smear for screening in women age 30 or older.[1] The workshop also provided an interim guideline for management after screening (FIGURE 1).

COMMONLY ASKED QUESTIONS

Incorporating HPV testing into primary screening provides a better risk assessment and an excellent negative predictive value, but also raises some new questions from patients and clinicians.

Why do we need to add HPV testing? Isn't the Pap test effective by itself?

The Pap smear is relatively insensitive and has to be repeated frequently to detect the disease in the general population. The problem with frequent testing is that it detects many cases of transient and minimal abnormalities that would not progress to cervical cancer. As a result, many women with abnormal Pap tests but no significant underlying pathology will undergo an invasive procedure to ensure that they do not have precancerous lesions.

Studies have also shown that almost one third of women with invasive cervical cancer have had one or more normal Pap tests or no abnormal Pap test during the previous 3 years.[47] The ALTS trial demonstrated that HPV testing can predict who really is at risk for CIN 2, CIN 3, or cancer and who is not. Most recent large clinical screening trials clearly demonstrated that combined HPV-plus-Pap testing has greater sensitivity for detecting these lesions than does Pap testing by itself.

TABLE 3

Recommendations for cervical cancer screening

HPV DNA testing for primary screening
- **ACS:** Yes, in combination with the Papanicolaou (Pap) test in women 30 years and older
- **ACOG:** Same as ACS recommendation
- **USPSTF:** Insufficient evidence to recommend for or against routine use

When to start screening
- **ACS:** Approximately 3 years after the onset of vaginal intercourse; no later than age 21
- **ACOG:** Same as ACS
- **USPSTF:** Same as ACS

Screening interval
- **ACS:** Annual with conventional Pap test or every 2 years using liquid-based ThinPrep until age 30. At or after age 30, Pap combined with HPV testing; if both negative, every 3 years
- **ACOG:** Annually in women < 30 years old; in women > 30 years old, same as ACS
- **USPSTF:** Every 3 years

When to stop screening
- **ACS:** Age 70 and older who have had three or more consecutive normal Pap tests
- **ACOG:** Individual basis
- **USPSTF:** Age 65 if she had adequate recent screening with normal Pap smears

Screening after hysterectomy
- **ACS:** If hysterectomy for a benign condition: no more screening; if hysterectomy was for precancer: continue screening for 10 years to achieve three consecutive negative Pap tests; if hysterectomy was for cancer, continue screening as long as the patient is in reasonably good health
- **ACOG:** If hysterectomy was for grade 2 or 3 cervical intraepithelial neoplasia, continue annual screening until three consecutive Pap smears are negative
- **USPSTF:** Same as ACS

ACS = American Cancer Society, ACOG = American College of Obstetricians and Gynecologists, USPSTF = United States Preventive Services Task Force

Furthermore, if both the Pap and HPV tests are negative, then the probability that CIN 3 or cancer is absent (the negative predictive value) is almost 100%.

Therefore, combined HPV-plus-Pap testing allows us to better identify women at risk of developing cervical cancer and to reassure women that "negative is negative" with a high degree of certainty.

Is it safe to screen women every 3 years?

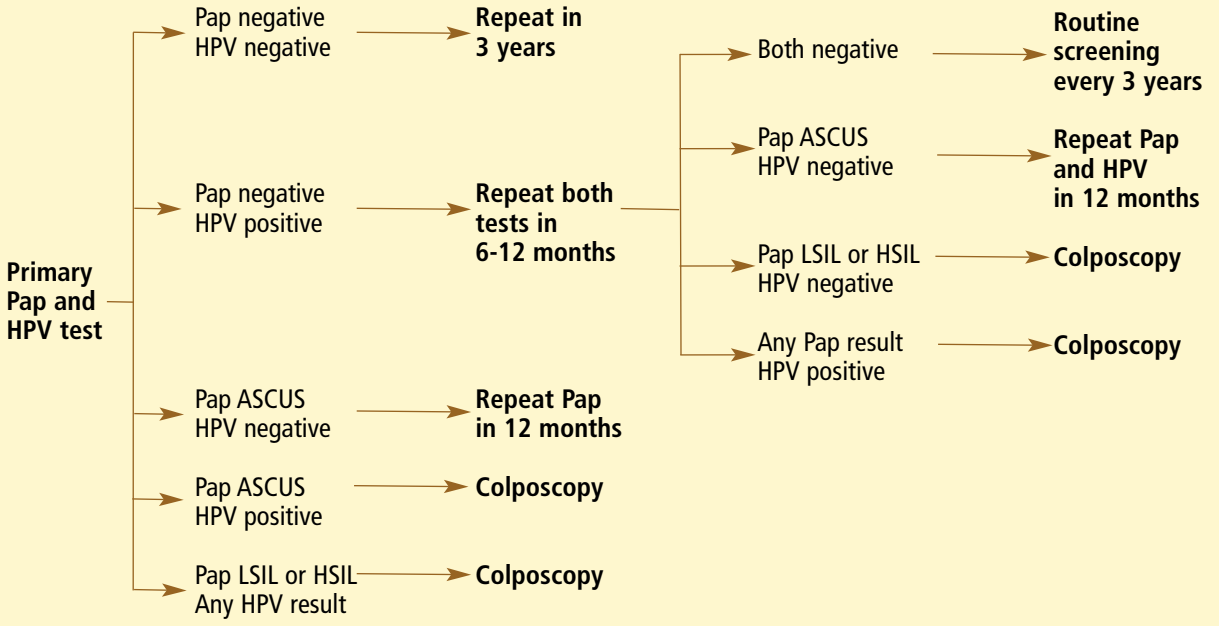
Some clinicians are concerned that even if a woman tests negative on both the HPV and Pap tests, she could subsequently acquire HPV from a new sexual partner and might be at risk of developing invasive cancer before her next screening in 3 years.

Many women acquire HPV, but few develop cancer

It is true that a woman can have a double-negative test today, acquire high-risk HPV tomorrow, and develop high-grade CIN within a few weeks or months. However, the transit time—the time from initial infection to the development of cervical cancer—usually exceeds 10 years.[48] Her high-grade CIN will be detected at her next screening, long before 10 years.

We have a similar screening model in clinical practice: colonoscopy. A negative colonoscopy at age 50 indicates a very low risk of colon cancer in the next 10 years, since the transit time is long. Therefore, clinicians should have a high level of comfort in promoting a longer screening interval in women over 30 who test negative on both the HPV and the Pap test.

Management algorithm after combined Pap and HPV testing



ASCUS = atypical squamous cells of undetermined significance, HPV = human papillomavirus (testing by Hybrid Capture 2), HSIL = high-grade squamous intraepithelial lesion, LSIL = low-grade squamous intraepithelial lesion, Pap = Papanicolaou smear

FIGURE 1

BASED ON WRIGHT TC JR, SCHIFFMAN M, SOLOMON D, ET AL. INTERIM GUIDANCE FOR THE USE OF HUMAN PAPILLOMAVIRUS DNA TESTING AS AN ADJUNCT TO CERVICAL CYTOLOGY FOR SCREENING. OBSTET GYNECOL 2004; 103:304–309.

Pap-negative but HPV-positive: Is it a 'false-positive'?

The combination of a positive HPV test plus a negative Pap test should not be considered a false-positive result, since HPV infection precedes the development of cytologic abnormalities.[49] If the HPV infection persists, the woman is at high risk of developing cervical cytologic abnormalities that will be detected on a subsequent Pap test.[50,51] Such patients should be followed closely.

'I am HPV-positive. How did I get it? Who gave it to me and when?'

HPV infection is indeed transmitted by sexual contact. Most likely, a woman with HPV infection acquired it from her sexual partner.[52,53] However, due to the latency of HPV infection, it is almost impossible to determine *when* she acquired it or from which partner. HPV infection certainly does not suggest infidelity or promiscuity.

Physicians need to provide appropriate counseling to women who test positive for HPV to avoid unnecessary anxiety and negative implications in personal relationships.

Should we test the male partners of women testing positive for HPV?

Screening male partners is not recommended at present.

Overall, little is known about the natural history of penile HPV infection.[54] Although men are believed to be vectors for HPV transmission, HPV DNA testing does not accurately reflect a man's HPV infection status or lifetime exposure to HPV even using highly sensitive methods.[55]

Only about one fifth of men whose wives are positive for CIN 3 test positive for penile HPV. Furthermore, the same HPV types are rarely identified in husbands and wives.[56]

FUTURE DIRECTIONS IN SCREENING AND PREVENTION

Biomarkers of cancer

Despite the value of HPV testing in women with ASCUS, only 77 of 611 women with ASCUS and HPV in the ALTS trial were subsequently found to have CIN 3. Clearly, many women underwent unnecessary colposcopy and biopsy.

Similarly, in routine primary screening, a positive HPV test does not predict the subsequent development of CIN 3 or cancer. The positive predictive value of HPV testing is poor.

Therefore, research is under way to identify markers that can be used to predict which lesion will regress and which will progress.

One of the most promising biomarkers for cervical cancer is $p16^{INK4A}$, a cyclin-dependent kinase inhibitor[57] that is strongly expressed in almost all cervical cancers. However, it is still not clear whether $p16^{INK4A}$ positivity can be used to distinguish which lesion will progress.[58]

HPV vaccine

To eliminate cervical cancer we will need not only effective screening, but also preventive strategies such as an HPV vaccine.

Recently, Koutsky et al[59] elegantly demonstrated the efficacy of HPV-16 vaccine in a clinical trial in 1,194 women. At 17 months, the incidence of persistent HPV-16 infection was 0 per 100 woman-years in vaccinated women, compared with 3.8 in a placebo group. No cases of HPV-16-related CIN occurred in the vaccinated group, vs 9 in the placebo group. This is a remarkable advance in cervical cancer prevention and a very powerful demonstration that cervical HPV infection and cervical cancer can be prevented by vaccination.

Ultimately, a vaccine against all oncogenic HPV strains will allow us to eradicate cervical cancer.[60]

REFERENCES

1. **Wright TC Jr, Schiffman M, Solomon D, et al.** Interim guidance for the use of human papillomavirus DNA testing as an adjunct to cervical cytology for screening. Obstet Gynecol 2004; 103:304–309.
2. **ACOG Practice Bulletin.** Clinical management guidelines for obstetrician-gynecologists. Number 45, August 2003. Cervical cytology screening (replaces committee opinion 152, March 1995). Obstet Gynecol 2003; 102:417–427.
3. **Saslow D, Runowicz CD, Solomon D, et al.** American Cancer Society guideline for the early detection of cervical neoplasia and cancer. CA Cancer J Clin 2002; 52:342–362.
4. **US Preventive Services Task Force.** Guide to Clinical Preventive Services: Periodic Updates. 3rd ed. Washington, DC: US Department of Health and Human Services; 2003.
5. **Wright TC Jr, Cox JT, Massad LS, Twiggs LB, Wilkinson EJ.** 2001 Consensus guidelines for the management of women with cervical cytological abnormalities. JAMA 2002; 287:2120–2129.
6. **Hutchinson ML, Agarwal P, Denault T, Berger B, Cibas ES.** A new look at cervical cytology. ThinPrep multicenter trial results. Acta Cytol 1992; 36:499–504.
7. **Lee KR, Ashfaq R, Birdsong GG, Corkill ME, McIntosh KM, Inhorn SL.** Comparison of conventional Papanicolaou smears and a fluid-based, thin-layer system for cervical cancer screening. Obstet Gynecol 1997; 90:278–284.
8. **Bernstein SJ, Sanchez-Ramos L, Ndubisi B.** Liquid-based cervical cytologic smear study and conventional Papanicolaou smears: a metaanalysis of prospective studies comparing cytologic diagnosis and sample adequacy. Am J Obstet Gynecol 2001; 185:308–317.
9. **Solomon D, Davey D, Kurman R, et al.** The 2001 Bethesda System: terminology for reporting results of cervical cytology. JAMA 2002; 287:2114–2119.
10. **Stoler MH.** New Bethesda terminology and evidence-based management guidelines for cervical cytology findings. JAMA 2002; 287:2140–2141.
11. **The Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesions Triage Study (ALTS) Group.** Human papillomavirus testing for triage of women with cytologic evidence of low-grade squamous intraepithelial lesions: baseline data from a randomized trial. J Natl Cancer Inst 2000; 92:397–402.
12. **Solomon D, Schiffman M, Tarone R.** Comparison of three management strategies for patients with atypical squamous cells of undetermined significance: baseline results from a randomized trial. J Natl Cancer Inst 2001; 93:293–299.
13. **Stoler MH, Schiffman M.** Interobserver reproducibility of cervical cytologic and histologic interpretations: realistic estimates from the ASCUS-LSIL Triage Study. JAMA 2001; 285:1500–1505.
14. **Lorincz AT, Reid R, Jenson AB, Greenberg MD, Lancaster W, Kurman RJ.** Human papillomavirus infection of the cervix: relative risk associations of 15 common anogenital types. Obstet Gynecol 1992; 79:328–337.
15. **Bosch FX, Manos MM, Munoz N, et al.** Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International biological study on cervical cancer (IBSCC) Study Group. J Natl Cancer Inst 1995; 87:796–802.
16. **Walboomers JM, Jacobs MV, Manos MM, et al.** Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999; 189:12–19.
17. **Herrero R, Hildesheim A, Bratti C, et al.** Population-based study of human papillomavirus infection and cervical neoplasia in rural Costa Rica. J Natl Cancer Inst 2000; 92:464–474.
18. **Thomas DB, Qin Q, Kuypers J, et al.** Human papillomaviruses and cervical cancer in Bangkok. II. Risk factors for in situ and invasive squamous cell cervical carcinomas. Am J Epidemiol 2001; 153:732–739.
19. **de Villiers EM.** Taxonomic classification of papillomaviruses. Papillomavirus Rep 2001; 12:57–63.
20. **Munoz N, Bosch FX, de Sanjose S, et al.** Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003; 348:518–527.
21. **Bosch FX, Lorincz A, Munoz N, Meijer CJ, Shah KV.** The causal relation between human papillomavirus and cervical cancer. J

Clin Pathol 2002; 55:244–265.
22. **Lancaster WD, Castellano C, Santos C, Delgado G, Kurman RJ, Jenson AB.** Human papillomavirus deoxyribonucleic acid in cervical carcinoma from primary and metastatic sites. Am J Obstet Gynecol 1986; 154:115–119.
23. **Boshart M, Gissmann L, Ikenberg H, Kleinheinz A, Scheurlen W, zur Hausen H.** A new type of papillomavirus DNA, its presence in genital cancer biopsies and in cell lines derived from cervical cancer. Embo J 1984; 3:1151–1157.
24. **Schlegel R.** Papillomaviruses and human cancer. Semin Virol 1990; 1:297–306.
25. **zur Hausen H, de Villiers EM.** Human papillomaviruses. Annu Rev Microbiol 1994; 48:427–447.
26. **Scheffner M, Romanczuk H, Munger K, Huibregtse JM, Mietz JA, Howley PM.** Functions of human papillomavirus proteins. Curr Top Microbiol Immunol 1994; 186:83–99.
27. **Arbeit JM, Munger K, Howley PM, Hanahan D.** Progressive squamous epithelial neoplasia in K14-human papillomavirus type 16 transgenic mice. J Virol 1994; 68:4358–4368.
28. **Greenhalgh DA, Wang XJ, Rothnagel JA, et al.** Transgenic mice expressing targeted HPV-18 E6 and E7 oncogenes in the epidermis develop verrucous lesions and spontaneous, rasHa-activated papillomas. Cell Growth Differ 1994; 5:667–675.
29. **Werness BA, Levine AJ, Howley PM.** Association of human papillomavirus types 16 and 18 E6 proteins with p53. Science Apr 6 1990; 248(4951):76–79.
30. **Scheffner M, Huibregtse JM, Vierstra RD, Howley PM.** The HPV-16 E6 and E6-AP complex functions as a ubiquitin-protein ligase in the ubiquitination of p53. Cell 1993; 75:495–505.
31. **Havre PA, Yuan J, Hedrick L, Cho KR, Glazer PM.** p53 inactivation by HPV16 E6 results in increased mutagenesis in human cells. Cancer Res 1995; 55:4420–4424.
32. **Munger K, Werness BA, Dyson N, Phelps WC, Harlow E, Howley PM.** Complex formation of human papillomavirus E7 proteins with the retinoblastoma tumor suppressor gene product. Embo J 1989; 8:4099–4105.
33. **Duensing S, Lee LY, Duensing A, et al.** The human papillomavirus type 16 E6 and E7 oncoproteins cooperate to induce mitotic defects and genomic instability by uncoupling centrosome duplication from the cell division cycle. Proc Natl Acad Sci USA 2000; 97:10002–10007.
34. **Richart RM, Cox JT, Kinney WK, Stoler MH.** Combined HPV and Pap testing: advances in risk assessment. Contemp Obstet Gynecol 2003; April:4S–16S.
35. **Nanda K, McCrory DC, Myers ER, et al.** Accuracy of the Papanicolaou test in screening for and follow-up of cervical cytologic abnormalities: a systematic review. Ann Intern Med 2000; 132:810–819.
36. **Bastian L, Datta S, Hasselblad V, et al.** Evaluation of cervical cytology, 5. Agency for Health Care Policy and Research. http://hstat.nlm.nih.gov/hq/hquest/db/local.epc.er.cyt/screen/doctitle/s/48139. Accessed February 25, 2003.
37. **Fahey MT, Irwig L, Macaskill P.** Meta-analysis of Pap test accuracy. Am J Epidemiol 1995; 141:680–689.
38. **Kulasingam SL, Hughes JP, Kiviat NB, et al.** Evaluation of human papillomavirus testing in primary screening for cervical abnormalities: comparison of sensitivity, specificity, and frequency of referral. JAMA 2002; 288:1749–1757.
39. **Zielinski GD, Snijders PJ, Rozendaal L, et al.** HPV presence precedes abnormal cytology in women developing cervical cancer and signals false negative smears. Br J Cancer 2001; 85:398–404.
40. **Allen KA, Zaleski S, Cohen MB.** Review of negative Papanicolaou tests. Is the retrospective 5-year review necessary? Am J Clin Pathol 1994; 101:19–21.
41. **Lorincz AT, Richart RM.** Human papillomavirus DNA testing as an adjunct to cytology in cervical screening programs. Arch Pathol Lab Med 2003; 127:959–968.
42. **Salmeron J, Lazcano-Ponce E, Lorincz A, et al.** Comparison of HPV-based assays with Papanicolaou smears for cervical cancer screening in Morelos State, Mexico. Cancer Causes Control 2003; 14:505–512.
43. **Belinson J, Qiao YL, Pretorius R, et al.** Shanxi Province Cervical Cancer Screening Study: a cross-sectional comparative trial of multiple techniques to detect cervical neoplasia. Gynecol Oncol 2001; 83:439–444.
44. **Wright TC Jr, Denny L, Kuhn L, Pollack A, Lorincz A.** HPV DNA testing of self-collected vaginal samples compared with cytologic screening to detect cervical cancer. JAMA 2000; 283:81–86.
45. **Petry KU, Menton S, Menton M, et al.** Inclusion of HPV testing in routine cervical cancer screening for women above 29 years in Germany: results for 8466 patients. Br J Cancer 2003; 88:1570–1577.
46. **Sherman ME, Schiffman M, Cox JT.** Effects of age and human papilloma viral load on colposcopy triage: data from the randomized Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesion Triage Study (ALTS). J Natl Cancer Inst 2002; 94:102–107.
47. **Sung HY, Kearney KA, Miller M, Kinney W, Sawaya GF, Hiatt RA.** Papanicolaou smear history and diagnosis of invasive cervical carcinoma among members of a large prepaid health plan. Cancer 2000; 88:2283–2289.
48. **Pinto AP, Crum CP.** Natural history of cervical neoplasia: defining progression and its consequence. Clin Obstet Gynecol 2000; 43:352–362.
49. **Wright TC Jr, Schiffman M.** Adding a test for human papillomavirus DNA to cervical-cancer screening. N Engl J Med 2003; 348:489–490.
50. **Kjaer SK, van den Brule AJ, Paull G, et al.** Type specific persistence of high risk human papillomavirus (HPV) as indicator of high grade cervical squamous intraepithelial lesions in young women: population based prospective follow up study. BMJ 2002; 325:572.
51. **Sherman ME, Lorincz AT, Scott DR, et al.** Baseline cytology, human papillomavirus testing, and risk for cervical neoplasia: a 10-year cohort analysis. J Natl Cancer Inst 2003; 95:46–52.
52. **Schiffman MH, Bauer HM, Hoover RN, et al.** Epidemiologic evidence showing that human papillomavirus infection causes most cervical intraepithelial neoplasia. J Natl Cancer Inst 1993; 85:958–964.
53. **Fairley CK, Chen S, Tabrizi SN, Leeton K, Quinn MA, Garland SM.** The absence of genital human papillomavirus DNA in virginal women. Int J STD AIDS 1992; 3:414–417.
54. **Schiffman M, Kjaer SK.** Natural history of anogenital human papillomavirus infection and neoplasia. J Natl Cancer Inst Monogr 2003; 31:14–19.
55. **Rubin MA, Kleter B, Zhou M, et al.** Detection and typing of human papillomavirus DNA in penile carcinoma: evidence for multiple independent pathways of penile carcinogenesis. Am J Pathol 2001; 159:1211–1218.
56. **Franceschi S, Castellsague X, Dal Maso L, et al.** Prevalence and determinants of human papillomavirus genital infection in men. Br J Cancer 2002; 86:705–711.
57. **Keating JT, Ince T, Crum CP.** Surrogate biomarkers of HPV infection in cervical neoplasia screening and diagnosis. Adv Anat Pathol 2001; 8:83–92.
58. **Sano T, Oyama T, Kashiwabara K, Fukuda T, Nakajima T.** Expression status of p16 protein is associated with human papillomavirus oncogenic potential in cervical and genital lesions. Am J Pathol 1998; 153:1741–1748.
59. **Koutsky LA, Ault KA, Wheeler CM, et al.** A controlled trial of a human papillomavirus type 16 vaccine. N Engl J Med 2002; 347:1645–1651.
60. **Crum CP, Rivera MN.** Vaccines for cervical cancer. Cancer J 2003; 9:368–376.

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