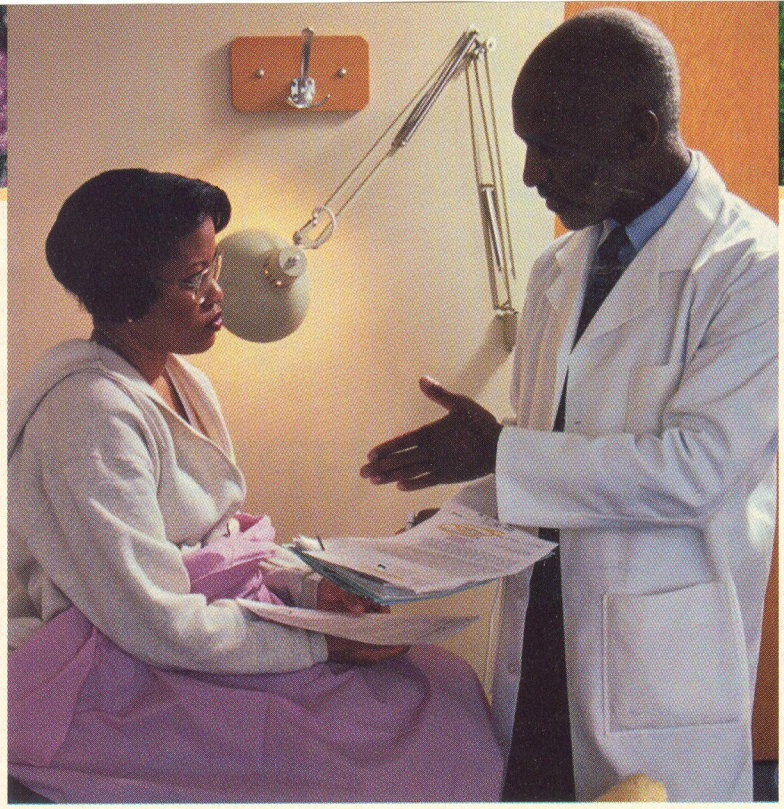

Early Detection of Cervical Cancer

New diagnostics identify HPV

BY ROBERT J. THOMAS

EVERY YEAR IN THE UNITED STATES, approximately 12,800 women are diagnosed with cervical cancer and 4800 die of the disease (*1*). It is second only to breast cancer as the most common cancer in women. On a worldwide basis, the problem is even more serious, affecting at least 400,000 women every year—of which 50% will die from the disease (*2*).

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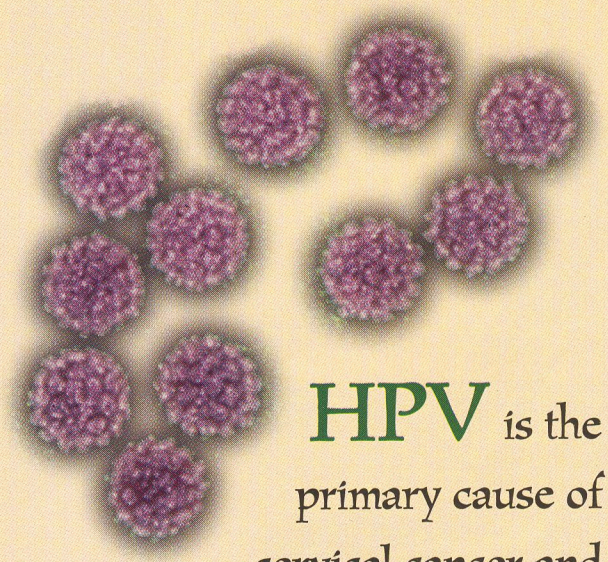
The major reason for cervical cancer's high mortality rate is that it is often dormant for 10–15 years and may not produce any clinical symptoms during this period. However, with early and regular screening for disease precursors, most cases of cervical cancer can be prevented or successfully treated. Traditional cytology screening methods, in place for more than 50 years, can only detect the disease in its later stages. These screening methods are often limited by a lab cytologist's interpretation of abnormal cell growth in the cervix. Recent improvements in liquid cytology have made the testing far more reliable, but the tests are still prone to error.



HPV is the primary cause of cervical cancer and is present in virtually all cases of the disease.

Identifying the disease in its early stages has driven researchers to focus on the underlying cause of cervical cancer and how it can be detected at the molecular level. This research has produced overwhelming evidence that virtually all cases of cervical cancer are caused by the human papilloma virus (HPV), making it perhaps the first cancer to be recognized as virally induced. Enormous strides have been made in the HPV–cervical cancer area, producing an accurate and reliable molecular-based DNA test for the virus. This work should lead to effective therapeutics and eventually a vaccine against the disease.

Traditional screening methods

Traditional screening for cervical cancer is done with a test called the Pap smear, named after George Papanicolaou, a scientist who pioneered the method in the early 1940s. The Pap smear tests for cervical cancer and a precancerous condition called cervical dysplasia. The sample is collected during a pelvic examination by scraping a small spatula and brush against the cervix and the lower portion of the uterus. The collected cells are smeared onto a slide, which is then sent to a lab where an experienced cytologist looks at the cells under a microscope. If no abnormal cells are seen on the slide, the result is considered normal.

Liquid cytology techniques have made the Pap smear more reliable. Instead of the traditional way of smearing the sample onto a slide, the cervical cell sample is rinsed into a vial of preserving liquid. The doctor then sends the vial to the laboratory, where an automated machine separates the cells and filters out the blood and mucus. A representative portion of the entire sample is then placed on a slide in a thin layer, so that it is clear and easy to read. This approach produces a homogenized, representative subsample.

The difference in the accuracy of results between the two preparation methods is quite significant. Instead of having to look through multiple cell layers, the cytologist has a clearer, unobscured view of the cervical cells. The result is that the liquid cytology method is much more effective than the conventional Pap smear method at detecting abnormal cells associated with low-grade lesions. In addition, fewer slides are classified as unreadable, but if the results are still unclear, the laboratory simply makes another slide from the sample, instead of repeating the entire sampling procedure.

The clinical cytology field lacked a consistent and accepted protocol for detecting and managing cervical disease until 1988, when the Bethesda System (*3*) for classifying cervical disease was introduced. The system has become the standard terminology used by labs in the United States to report cervical cytological abnormalities. It was developed to reflect the advanced understanding of cervical disease and to ensure correlation with histological terminology. The five primary categories used are

- negative or within normal limits,
- atypical squamous cells of undetermined significance,
- low-grade squamous intraepithelial lesions,
- high-grade squamous intraepithelial lesions, and
- carcinoma.

In the United States, physicians base follow-up testing and treatment decisions on the Bethesda System. Women with normal Pap smears continue to receive annual Pap smear exams. Women with abnormal Pap smears are referred for a colposcopic examination (a procedure that uses a special microscope to view the cervix). If the patient has any suspected lesions, a gynecologist usually takes biopsy samples during the colposcopy.

Although patient management protocols are well defined for the normal and abnormal Pap smear results, the follow-up for a patient with atypical squamous cells of undetermined significance is more challenging. In the medical community, this category is known as "I don't know", because the laboratory is unsure about the status of the Pap smear. Women who receive this result are often treated as if they have abnormal Pap smears, even though only an estimated 25–35% of them actually have cervical disease. The remaining 65–75% of these women go through needless physical and emotional stress.

A molecular diagnostic approach

The use of the Pap test as a primary screen for cervical cancer over the last 50 years has reduced the incidence of this disease by 75% in the United States. Yet despite this enormous succe each year, thousands of American women unnecessarily di cervical cancer. Most instances of cervical cancer in the United States occur in women who have not had a Pap test within the last five years, which reinforces the importance of regular screening.

The problem with the Pap smear is that it does not confirm the

presence or absence of HPV. The presence of the virus could be used as an early indication of disease potential. Because the Pap test can only detect clinical evidence of cervical disease, molecular-based diagnostic tools are being used more frequently to detect the virus before abnormal cell growth can be observed.

Over the past decade, clinical virology studies have categorically confirmed that HPV is the primary cause of cervical cancer and is present in virtually all cases of the disease (*4*). There are more than 70 kinds of HPV, but only 13 are known to be associated with cervical cancer. HPV is one of the most common sexually transmitted diseases in the United States, and it is estimated that up to 80% of women will get HPV at some time during their lives. However, only those women who have persistent infections with one or more of the cancer-causing kinds of HPV are at risk of developing cervical cancer. Although many women become infected in their early twenties, only a few of these women with HPV will progress to develop cervical cancer. This is because of a combination of the natural defenses of young women and the availability of early treatment following an abnormal Pap test. These factors might explain why older women are the fastest growing segment of the population for cervical abnormalities. In fact, 25% of all cervical cancers are in women older than 65. There is growing scientific evidence to suggest that the ability to identify the presence of high-risk types of HPV is a key factor in combating this disease at the molecular level.

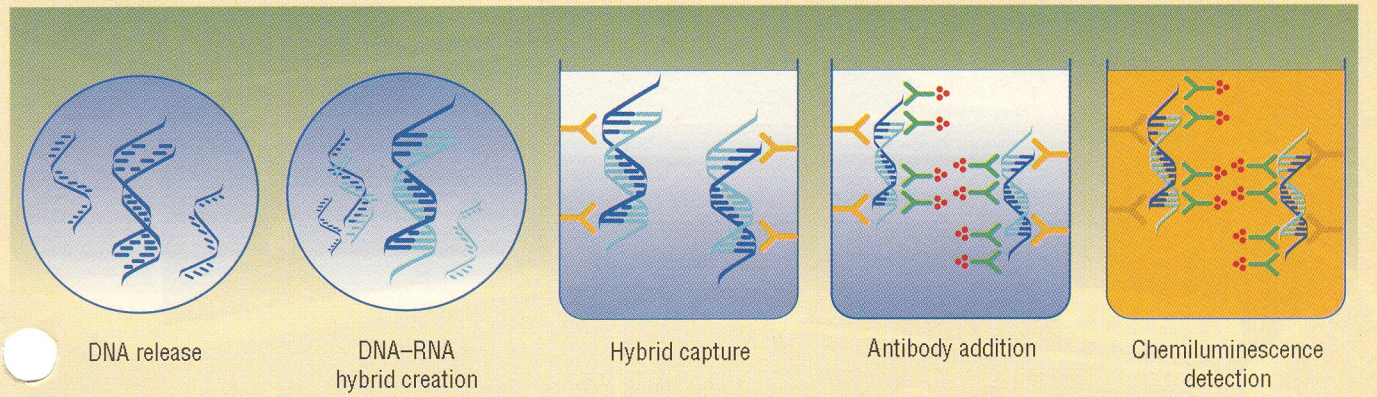


Figure 1. The Hybrid Capture System is a signal amplification assay that uses antibody capture and signal detection. Specimens are combined with a basic solution that disrupts the virus and releases target DNA. The DNA then combines with RNA probes, creating a DNA–RNA hybrid. Hybrids are captured onto a solid phase that has been coated with antibodies specific for the hybrids. Captured hybrids are detected with antibodies bound to alkaline phosphatase. Upon release by alkaline phosphatase, a dioxetane substrate produces chemiluminescence that is measured with a luminometer.

An FDA-approved test is now available to determine the presence of the types of HPV that cause cervical cancer. Developed by Digene Corp., the Hybrid Capture technology relies on the principle of signal amplification of a hybrid species produced by RNA probes complexing with the DNA of the 13 cancer-causing kinds of HPV. This RNA–DNA hybrid is then "captured" with specific antibodies and quantified using chemiluminescent signal detection (Figure 1). The benefit of the HPV test is that when used in conjunction with the Pap smear, it can categorically confirm whether patients with abnormal results are at risk of developing cervical cancer. Unlike the Pap smear alone, there is no question of uncertainty.

According to an article in the *Journal of the American Medical Association* (*5*), HPV testing can help identify patients at high and low risk for developing cervical cancer. Finding DNA infected with high-risk HPV suggests the presence of a precancerous lesion, particularly in women who have an abnormal or atypical Pap test. These high-risk patients should have a colposcopy and surgical treatment if necessary. Conversely, finding a low-risk HPV type in a patient with a negative Pap test means those patients can be managed less aggressively. Women in this low-risk category can generally be screened every six months, which avoids the emotional trauma of overtreating women with mild cervical abnormalities.

Research continues to uncover more evidence to support using molecular-based HPV testing for primary cervical cancer screening. A joint statement recently issued by the World Health Organization and the European Research Organization on Genital Infection and Neoplasia at an international meeting (*6*) stated that, based on trials involving more than 35,000 women from 9 countries, HPV testing showed 95–100% sensitivity for high-grade cancer precursors compared with 40–85% for traditional cervical cytology (Pap test). In fact, the statement strongly suggested that HPV testing should be adopted as the primary screening method for women over 30.

Treatments

If HPV is detected early enough, virtually all cervical cancer is preventable. Only when the disease progresses to the invasive stage will it require treatment. The most common form of treatment is removal of the diseased tissue by conventional surgery, laser excision, or cryosurgery, often followed by chemotherapy or radiation. In some extreme cases, a hysterectomy might have to be performed. But these measures address the effects of the disease, not its cause. It is clear that the only way cervical cancer is going to be cured is through effective therapeutics against HPV.

The major pharmaceutical companies that are working on HPV therapies are looking for ways of triggering the immune system to fight the virus. Among the most promising ways being investigated is the use of a new class of drugs called immune response modifiers, which stimulate the production of certain kinds of hor-

monelike proteins, such as interferons, to mimic the body's natural response to a viral infection. Preclinical studies of these drug candidates, which can be applied as creams for HPV infections, suggest that they do not induce direct antiviral activity, but work by in vivo activation of the immune system by the proteins.

Several pharmaceutical companies have HPV vaccine development programs under way. The two most common approaches are immunotherapeutic and prophylactic vaccines. Immunotherapeutic vaccines treat an HPV infection by using the elements of the virus or a recombination form of a protein associated with the virus and an unrelated protein to stimulate an immune system response. Prophylactic vaccines use combinations of proteins to make viruslike particles to stimulate the immune system to recognize and destroy the virus before infection. However, the drawback to vaccine development is that because the natural progression rate of cervical cancer is 10–15 years, clinical trials will require substantial time to evaluate viability.

Screening programs are currently the most effective treatment to prevent cervical cancer. Even though the Pap test has significantly reduced the rate of cervical cancer, HPV testing could further reduce the rate. These programs will help detect the disease and identify women at risk for it in its early stages, when therapeutics have a higher probability of success. The superior negative predictive value of HPV testing will also permit longer screening intervals, in addition to helping women with equivocal Pap test results.

Only by more screening and focusing on at-risk women can cervical cancer be treated more efficiently to eventually reduce the overall cost to our overburdened healthcare system. Successful vaccines against HPV, along with effective therapeutics, will help to eradicate cervical cancer in this century.

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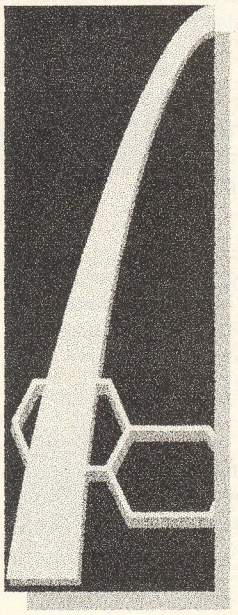

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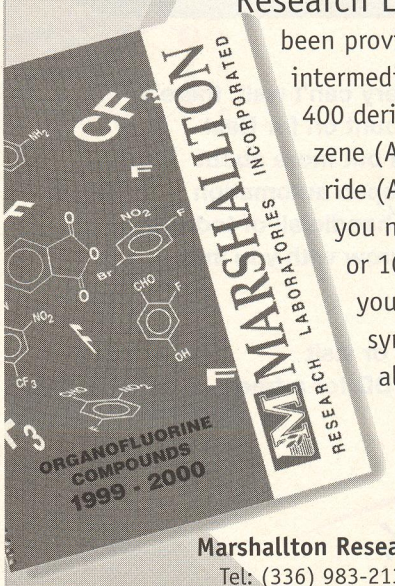

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