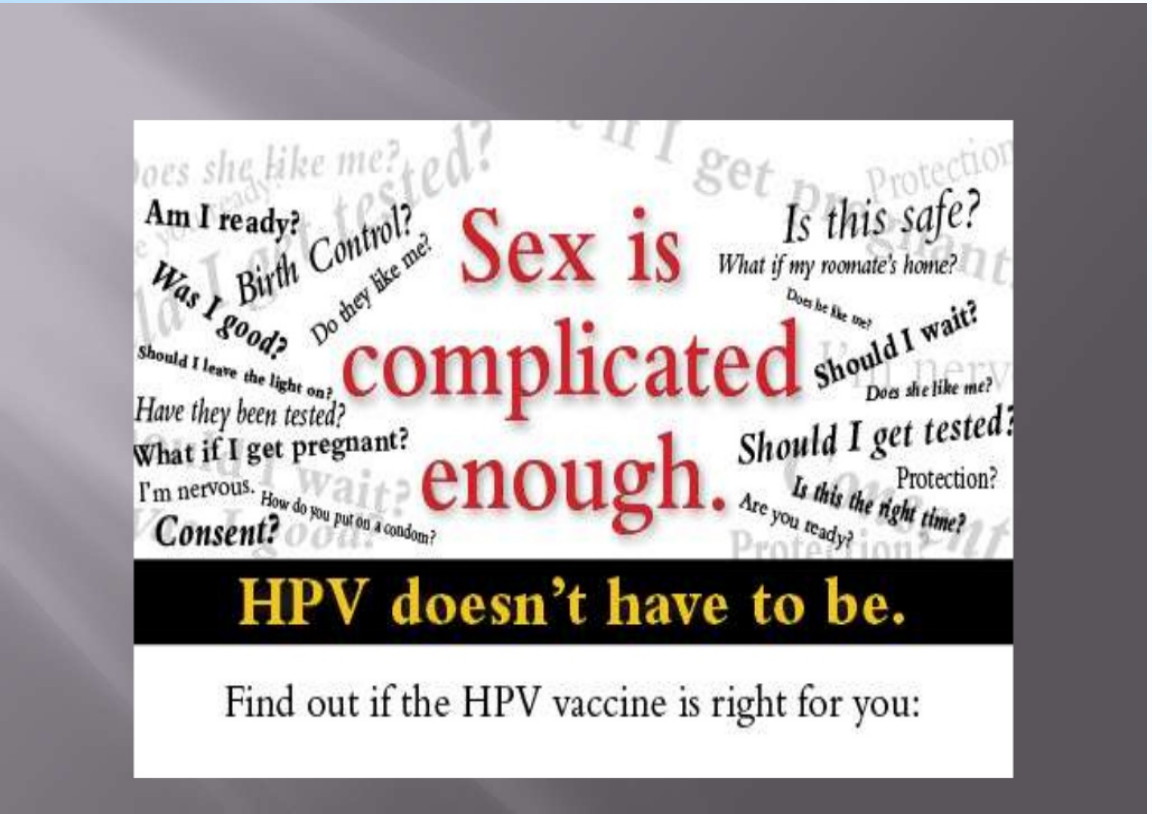
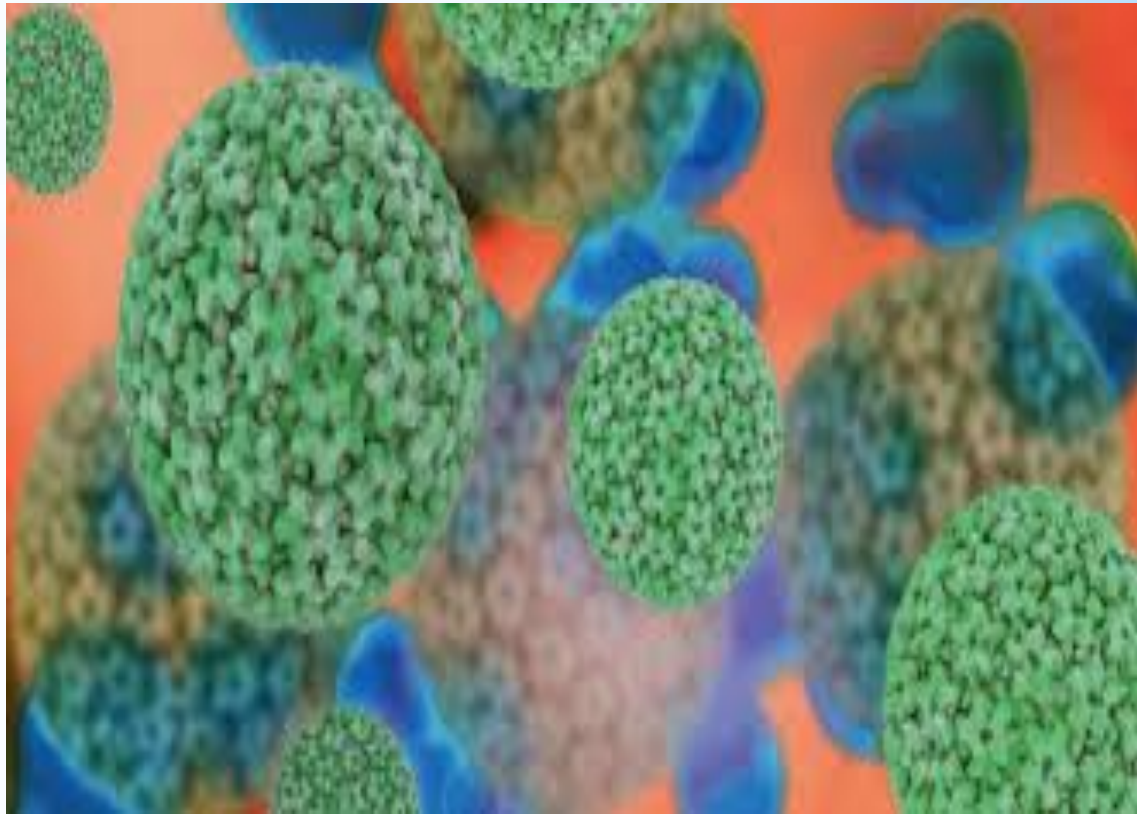
بسم الله الرحمن الرحيم

* Cervical cancer screening tests: Techniques for cervical cytology and human papillomavirus testing





* INTRODUCTION

— Cervical cancer screening detects **precancerous** changes of the cervix (eg, cervical dysplasia), often making treatment possible

before cervical cancer develops.

Screening uses human papillomavirus (HPV) testing, cervical cytology (Pap test), or a combination of the two tests (eg, "cotesting").

* HOW TO OBTAIN A SAMPLE

— Cell samples for cervical cytology and HPV testing are obtained during the speculum examination. With certain types of Pap

tests (eg, ThinPrep), the same specimen can be used for analysis of both cytology and HPV;

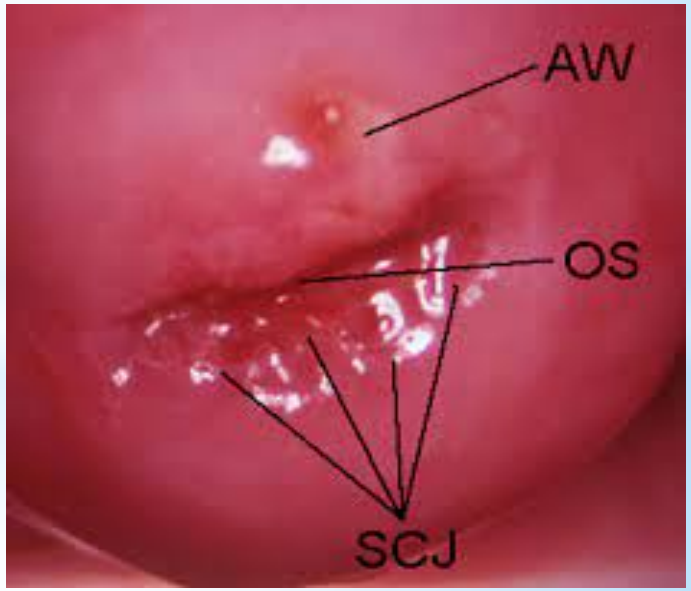
alternatively, some tests (eg, SurePath) require that separate specimens be obtained.

* Specimens for cytology

– There are two methods for preparing a specimen for cervical cytology

For both methods, cells are obtained from the external surface of the cervix (*ectocervix*) and the cervical canal (*endocervix*) to evaluate the transformation zone (SCJ).

SCJ : The area at **greatest risk** for neoplasia.

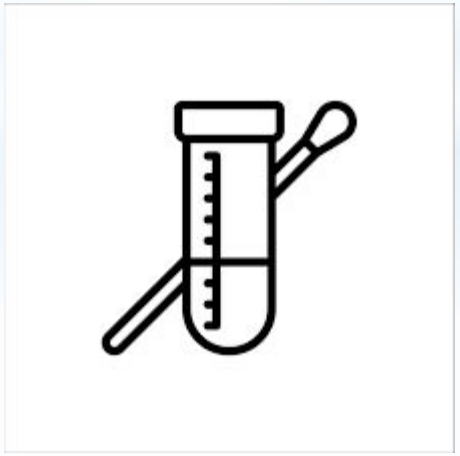


* Collection device

—A spatula and a separate endocervical brush (endocervical cells) than when only a spatula is used.

It is also slightly **better** for detecting any grade of cervical intraepithelial neoplasia (*CIN*) than the single broom device.

Cotton tipped swabs should be avoided because they collect fewer endocervical cells and do not detect CIN as well as other devices .



* Sample collection

– *To obtain cells from the cervix*:

Use the **spatula** to circumferentially scrape the *ectocervix* (for liquid-based samples, use a plastic rather than a wooden spatula; wood or plastic is fine for conventional smears).

Sampling the ectocervix before the endocervix will minimize **bleeding** during sample collection.

Obscuring blood in the sample interferes with interpretation of conventional Pap smears more than with liquid-based specimens.

Insert the endocervical brush into the endocervix so that the bristles nearest the examiner are inserted to the level of the external cervical os. Rotate the brush **180 degrees** to obtain a sample.

- Alternatively, if a *broom* is used, insert the central bristles into the endocervix with the outer bristles in contact with the ectocervix. Rotate the broom in the same direction for *five turns*.

- Other devices on the market (eg, SpiraBrush, SoftBiopsy) have yet to be adequately studied with respect to safety and efficacy.

- In patients at high risk for vaginal cancer because of in utero *diethyl stilbestrol* exposure, additional samples from the anterior and posterior fornices should be obtained.

* Preparation methods

There are two methods for preparing a specimen for cervical cytology: the conventional Pap smear and the liquid-based, thin layer preparation

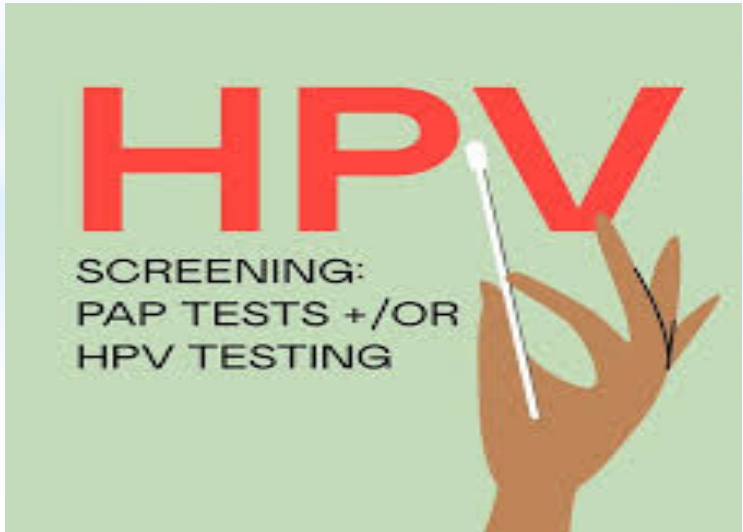
For **conventional Pap smears**, the ectocervical spatula is smeared and the endocervical brush is rolled uniformly onto a single slide promptly after obtaining the specimens .

The slide is then rapidly fixed to avoid air-drying; the usual fixativesare either ethyl ether plus **95** percent ethyl alcohol or **95** percent ethyl alcohol alone.

If spray fixatives are used, the spray should be held at least **10 inches** away from the slide to prevent disruption of cells by the propellant.

For **liquid-based thin layer cytology**, the collecting device is placed into a liquid fixative solution and vigorously swirled or rotated **ten times** in the solution .

When the liquid is processed by the cytology laboratory, loose cells are trapped onto a filter and then plated in a monolayer onto a glass slide.



For both methods, cells are obtained from the ectocervix and the cervical canal (endocervix) to evaluate the transformation zone (squamocolumnar junction), the area at greatest risk for neoplasia.

An advantage of some liquid-based systems is the ability to use a single specimen for cytology and testing for HPV.

With conventional smears, a separate HPV test specimen has to be obtained.

The interpretation of cytologic smears is subject to considerable interobserver variability, particularly in the case of nondiagnostic squamous and glandular atypias (atypical squamous cells of undetermined significance and atypical glandular cells of undetermined significance) .

In some settings, cytotechnologists and/or automated slide interpretation devices perform initial review of the cytology slides to identify a subgroup of slides for subsequent evaluation by a cytopathologist. This subgroup consists of slides with specific abnormal characteristics.

* Sample processing

One example of an automated slide interpretation system is the ***ThinPrep* Imaging** System, which is approved by the (FDA) for primary screening of slides.

This system uses programmed algorithms to review each slide for areas of most concern.

If abnormalities are found, the whole slide is reviewed by a cytopathologist.

In one study, use of this device increased detection of (**HSIL**) by *38 percent* and (**LSIL**) by *46 percent* compared with manual screening .

In another study, use of the imager resulted in fewer unsatisfactory slides than with conventional cytology (1.8 versus 3.1 percent) and better detection of HSIL .

Although promising, the clinical effectiveness of automated systems and their role in cervical cancer screening have not been definitively established.

In the United States, quality assurance regulations require that laboratories rescreen 10 percent of randomly selected cervical cytology smears that were originally interpreted as negative .

Manual rescreening of all negative cytology smears is time consuming, although rapid manual rescreening (**30 to 120 seconds per slide**) is feasible and practiced in the United Kingdom and elsewhere.

Standardized terminology for reporting cervical cytology results was introduced with the Bethesda System in 1988, which was last revised in 2014.

* HPV testing

HPV testing identifies oncogenic (ie, high-risk) HPV subtypes that are associated with cervical cancer .

The various HPV testing systems are approved for either primary HPV testing (without cervical cytology) or **cotesting** (with cervical

cytology) .

Tests that are FDA-approved for cotesting should only be used alongside cervical cytology and **not** be used for primary HPV testing .

* Cervical testing

– Specimens for HPV testing can be collected from the endocervix using a cervical spatula or cervical brush, which is then placed

in HPV test transport medium .

With some LBC sampling systems, the same specimen can be used for HPV testing and cytology.

In resource-limited settings, self-collection of an HPV sample by the patient is being used. Patients can collect samples from the **vagina** using a *tampon*, *Dacron* or *cotton swab*, *cytobrush*, or *cervicovaginal lavage*.

* Other methods

— **Urine testing** for HPV has been proposed, but is not clinically available. This testing method may have utility if HPV testing alone (without cervical cytology) is used for cervical cancer screening.

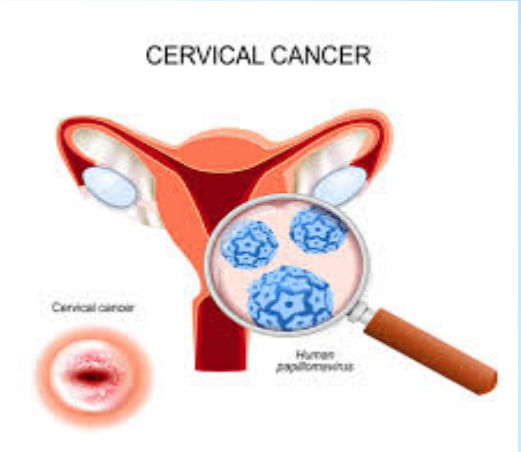
The efficacy of urine testing was evaluated in a meta-analysis of 14 studies including 1443 patients .Most studies used commercial polymerase chain reaction methods, and cervical testing results were used as the reference standard.

For detection of high-risk HPV, the sensitivity was 77 percent and specificity was 88 percent. For detection of HPV ***16 and 18,*** sensitivity was 73 percent and specificity was 98 percent.

Sensitivity was statistically significantly higher when urine samples were collected as first void compared with random or midstream.

Such a test may have potential in large research studies or as an alternative test where routine **cervicovaginal** examinations are not economically feasible or less likely to be performed due to ***cultural barriers***.

* Additional tests



— Additional testing that may be performed during examination of the cervix includes:

Gonorrhea, chlamydia, and trichomonas

— It is common practice to collect the cytology sample before testing for cervical infection, if indicated.

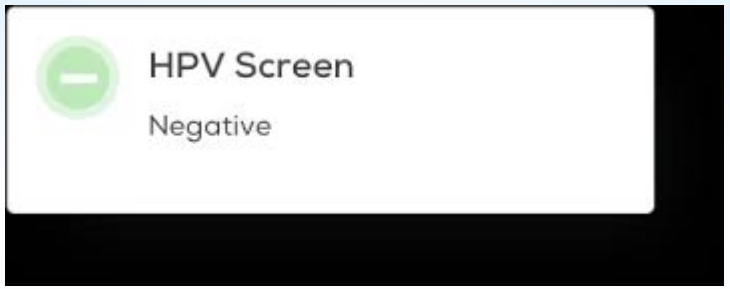
However, there is no evidence that the order in which the samples are obtained affects cytology results .

LBC systems allow testing for cytology, HPV, gonorrhea, chlamydia, and trichomonas from a ***single specimen***.

* Biopsy of visible lesions

— During Pap testing, any lesion that is raised, friable, or has the appearance of **condyloma** should be biopsied, regardless of previous cytology results or other risk factors for cervical cancer .

*The only *visible lesions* that do not require biopsy are Nabothian cysts and only when this diagnosis is confirmed by an experienced examiner.



* Anatomic barriers

In some patients, the cervix is difficult to visualize on pelvic examination. Factors that may make visualization difficult include:

*High BMI

* Prior cesarean section.

* A uterus that is sharply anteverted or retroverted.

*Obliteration of the vaginal fornices (from menopause-induced vaginal atrophy, prior pelvic radiation, or vaginal graft-versus host disease).

If the clinician **cannot see the cervix**, options include the following:

Use a longer ***Graves*** or Pederson speculum to reach the vaginal apex; press the speculum along the posterior vaginal wall until the apex is reached and then open the speculum slowly.

- Perform a bimanual examination to palpate the cervix and identify its location.

In patients with obliteration of the vaginal fornices, palpation often allows the examiner to differentiate the firm cervical tissue from the surrounding vaginal walls.

Lubricant is sometimes avoided as it can interfere with the ability to analyze the Pap specimen.

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Improve visualization by optimizing the patient's position.

In dorsal lithotomy, the following modifications can be used:

* Ensure that the patient's legs are suficiently abducted. The patient may need to move toward the examiner.

Care should be taken if the patient has knee or hip mobility issues.

* Elevate the sacrum by placing an object (bedpan, folded up sheet or towel) under the patient's hips.

* Confirm that the patient has a cervix (some patients who have undergone a total hysterectomy do not give an accurate surgical history).

* In patients with **cervical stenosis**, it may be difficult to obtain an endocervical sample, thus resulting in an insufficient result.

When it is difficult to insert the sampling device into the endocervix, one of the following techniques may facilitate collection of an endocervical sample:

- Perform Pap testing during menses. Menstrual blood often slightly dilates the cervix.
- grasp the anterior or posterior lip of the cervix with a single-tooth **tenaculum**. Applying gentle traction will stabilize the cervix and may provide enough counter-tension to insert a sampling device into the external cervical os.

- Administer a ***para- or intracervical block***, and use small mechanical dilators to dilate the cervix.

* SAMPLING CHALLENGES

There is perception that any action that may remove cells from the cervix (eg, prior Pap sampling, cervical cultures, swabbing)

will impair Pap test cellularity, and thus compromise efficacy for cervical cancer screening.

* Menses or other genital tract bleeding

— Historically, patients planning to have screening cytology for cervical cancer have been advised to **avoid** testing during menses or other genital tract bleeding.

We suggest performing rather than deferring the test, unless the blood cannot be cleaned from the cervix. Cleaning the cervix with a large cotton swab will remove obscuring blood and appears to have a minimal or no effect on sample cellularity .

If there is obscuring blood, conventional Pap smears are more likely to be ***unsatisfactory*** for interpretation than liquid-based methods because liquid-based techniques filter out red blood cells.

* in the Netherlands in which over 100,000 patients who reported having regular menstrual cycles were screened for cervical cancer using the *conventional Pap smear* . The rate of *unsatisfactory* smears was *23 percent* during cycle days 0 to 3 versus 2 percent for the remainder of the cycle.

* For liquid-based Pap tests, timing during the menstrual cycle does not appear to have a clinically significant effect on cytologic results. This was illustrated by a large study in which 5060 patients with initial cytology showing (ASC-US) or (LSIL) had over 20,000 liquid-based Pap tests.

* The phase of the menstrual cycle did not have a significant effect on the rate of unsatisfactory specimens. Although the detection of LSIL or more severe abnormalities was slightly higher in the mid- versus early or late cycle (mid-cycle: 20 percent, early and late cycle: 18 percent), this difference is unlikely to be clinically significant.

* HPV testing results are not affected by ***bleeding***, although some data suggest that detection of high risk varies with the phase of the menstrual cycle .

* Interval between Pap tests

— A Pap test may need to be repeated after a brief interval if the sample is unsatisfactory or at the time of colposcopy. A short interval between Pap tests (***15 to 30 days***) does not appear to affect sensitivity for detection of cervical neoplasia .

The concern about a short interval between Pap tests is based on the hypothesis that previous scraping for cytology will remove the most superficial layer of cervical cells, where a potential abnormality is most likely to occur. It will then take a period of time (generally estimated as up to two months) for the superficial cells to regenerate .

Thus, if sampling is performed too soon, the underlying cells may be sampled and appear normal, yielding a false negative test.

The most informative study of the optimum interval between Pap smears evaluated liquid-based Pap tests and HPV testing results in 5055 patients with initial Pap tests that showed either ASC-US or LSIL .

Sensitivity of repeat specimens was assessed by comparing cytology with histology in patients with cervical intraepithelial neoplasia 3 or carcinoma.

The interval from initial to repeat Pap test in these patients was ***8 to 184 days***. Adequacy of the sample did not appear to be affected, since cellularity of the sample and HPV viral load did not vary with Pap intervals.

In addition, Pap test interval (15 to 120 days) did not signifficantly affect the finding of abnormal cytology. In fact, the likelihood of a Pap result of LSIL was higher after a shorter versus a longer interval (≤30 days [30 percent] versus >120 days [20 percent]); this likely represents regression of LSIL with time.

* Gel lubricants and other contaminants

Contaminants, such as gel lubricant, *vaginal discharge*, *semen*, *spermicide*, or *intravaginal medications*, have been thought to affect cervical sampling.

On a conventional smear, the concern is that these may make the smear thick and difficult to read.

If large amounts of vaginal contaminants are present, the discharge can be *removed* gently with a *large cotton* swab without interfering with cytology results.

Gel lubricant on the speculum or on an examiner's hand before a Pap test is performed is commonly thought to interfere with the results of cervical cytology.

Some lubricants, particularly those that include *carbomers* or *carbopol polymers* may interfere with sample interpretation.

Clinicians should check with their cytology laboratory for approved lubricants.

In general, studies have not shown an adverse impact of lubricants on cervical cytology interpretation .

However, in our experience, a sample is often returned by the laboratory with a note regarding difficulty in interpretation because of lubricant.

As discussed above, testing for Neisseria gonorrhea and Chlamydia trachomatis cervical infection is often performed concurrently with cervical cytology.

Many clinicians avoid use of gel lubricants prior to testing for these bacteria, since some lubricants are bacteriostatic (eg, those containing ***chlorhexidine gluconate***).

There are no data regarding the effect of discharge, semen, or intravaginal medications on cervical cytology interpretation.

Studies of use of *nonoxynol-9 spermicide* have had conficting results regarding cervical cytology changes .

The spermicide nonoxynol-9 is not active against HPV, but detergents, such as sodium dodecyl sulfate (SDS), do inactivate HPV, and a spermicide/SDS combination could be useful in preventing HPV transmission

* Vaginal intercourse, douching, and tampon use

- Patients are typically advised to refrain from vaginal activities (eg, douching, tampon use, sexual intercourse) during the **48** hours prior to a Pap test.

- Advising patients to avoid vaginal activities may be cumbersome to clinicians and make timely scheduling of Pap tests difficult for patients.

- Vaginal intercourse and its effect on cervical cytology have not been studied. However, a study of HPV testing in which patients performed self-sampling with synthetic polyester swabs or a tampon found no effect on HPV detection when vaginal intercourse occurred within 48 hours of sampling .
- This was a small study, and the data cannot be generalized to clinician-performed testing in patients who have had recent vaginal intercourse prior to Pap testing. Although it is possible that HPV detected in cytology samples following recent sexual activity could be derived from the male partner, this should not alter the standard management algorithm.

Douching did not affect sensitivity of HPV testing in a study of 132 patients (sensitivity was 98 percent before douching; 96 percent

after douching); results of the effect of douching on cytology results were not reported .

More data are needed to address the effect of douching on cervical cytology and HPV testing.

There are no data regarding tampon use or barrier contraception and cervical cytology or HPV testing.

* SUMMARY AND RECOMMENDATIONS

- Cervical cancer screening tests detect cellular changes or infection with types of human papillomavirus (HPV) that predisposepatients to invasive cervical cancer.

- Conventional cervical smears are performed by smearing the specimen on a slide. With liquid-based methods, the specimen is placed into a liquid fixative solution. Both methods are referred to as cervical cytology or a Pap test.

- Several types of collection devices can be used for cervical cytology sampling.

- HPV testing detects strains of the virus that are associated with a high risk of cervical neoplasia. There is no commercially available test for detection of low-risk HPV strains. The various HPV testing systems are approved for either primary HPV.

- testing (without cervical cytology) or cotesting (with cervical cytology).
- For patients with vaginal bleeding, cleaning the cervix with a large cotton swab prior to performing a Pap test will remove obscuring blood and appears to have a minimal or no effect on sample cellularity.
- If cervical cytology needs to be repeated (eg, a previous test was unsatisfactory), a short interval of 15 to 30 days between tests does not appear to affect diagnostic results.
- Sexual intercourse, douching, and tampon use may remove the most superficial layer of cervical cells. However, it appears that removal of cells by these activities or by swabbing (to remove blood or discharge) does not diminish the ability to diagnose cervical abnormalities or HPV infection.
- Use of some lubricants before performing a Pap test may interfere with results of cytology


Thank you