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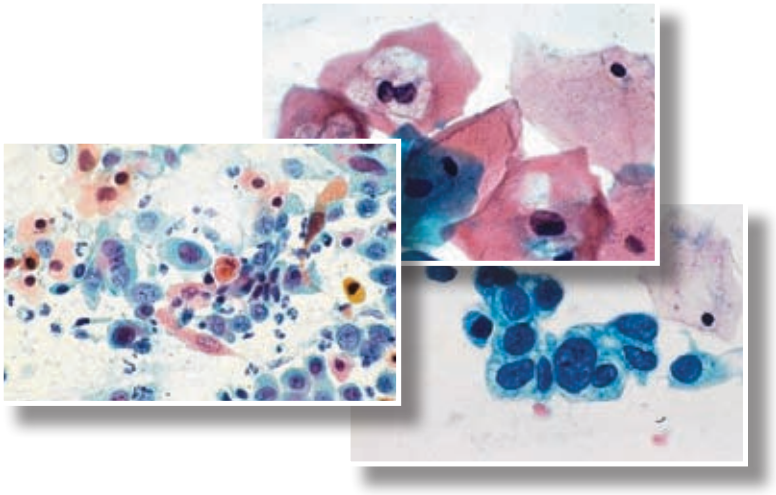
Cytopathology GYN Reference Guide



WELCOME

This pocket reference is intended as a guide for practice by health care providers who are responsible for conducting the screening examination and appropriate follow-up activities. The recommendations herein are not intended to be a substitute for sound professional judgement by providers in individual cases, nor are they intended to replace or alter the fundamental responsibilities inherent in the provider-patient relationship.

The UWHC Cytopathology
Division of the Department of Pathology
ph 608-263-3205 fax 608-263-6453

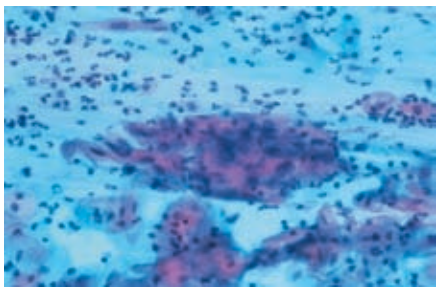
CONTENTS

CYTOLOGY REQUISITION FORMS

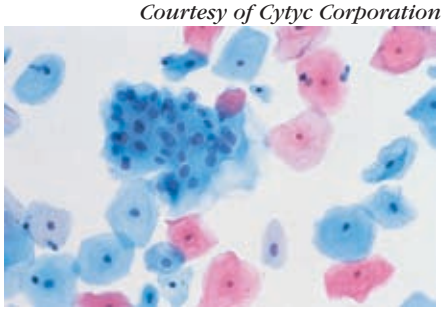
- When ordering a Pap test on paper requisition - use form # 1280520A.
- Complete the following required fields (shaded):
  - Diagnostic, Screening (low or high risk) box
  - Specimen Type
  - Specimen Site (ECC brushing is a replacement for ECC curettage)
  - LMP (last menstrual period)
- When ordering via HealthLink, no paper is necessary. Order screens will solicit required info.
- Required fields for electronic and paper orders are driven by regulatory requirements.

WHY USE THE THINPREP® PAP TEST™?

Courtesy of Cytyc Corporation



CONVENTIONAL SMEAR



THINPREP® PAP TEST™

Improves Sample Quality and detection of abnormalities over conventional pap smear

METHOD

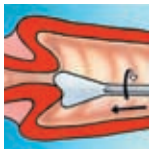
Meta-analysis of 25 articles published between 1990-2000 comparing cytology and specimen adequacy of the two tests for 67,484 patients

FINDINGS

Odds ratios favored the ThinPrep® Pap Test™ over the conventional Pap smear in regard to the diagnosis of LSIL and HSIL; adequacy was improved in the ThinPrep Pap Test group in all trials

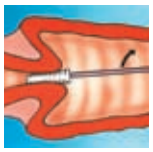
"...Thin Prep appears to be a superior method of evaluating uterine cervix cytologic abnormalities with regard to low-grade and high-grade lesions, as well as a better means of obtaining specimen adequacy for improved evaluation." Bernstein SJ, Sanchez-Ramos, Ndubisi B. (AmJ Obstet Gynecol. 2001; 185: 308-317.)

ThinPrep® Pap Test™ Quick Reference Guide -
Endocervical Brush/Spatula Protocol



OBTAIN an adequate sampling from the ectocervix using a plastic spatula.

RINSE the spatula as quickly as possible into the PreservCyt® Solution vial by swirling the spatula vigorously in the vial 10 times. Discard the spatula.



OBTAIN an adequate sampling from the endocervix using an endocervical brush device. Insert the brush into the cervix until only the bottommost fibers are exposed. Slowly rotate 1/4 or 1/2 turn in one direction.
DO NOT OVER-ROTATE.

RINSE the brush as quickly as possible in the PreservCyt Solution by rotating the device in the solution 10 times while pushing against the PreservCyt vial wall. Swirl the brush vigorously to further release material.
Discard the brush.

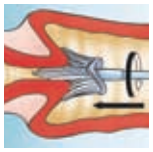
TIGHTEN the cap so that the torque line on the cap passes the torque line on the vial.

RECORD the patient's name and ID number on the vial ....the patient information and medical history on the cytology requisition form

Courtesy of Cytyc Corporation

ThinPrep® Pap Test™ Quick Reference Guide -

Broom-Like Device Protocol



OBTAIN an adequate sampling from the cervix using a broom-like device. Insert the central bristles of the broom into the endocervical canal deep enough to allow the shorter bristles to fully contact the ectocervix. Push gently, and rotate the broom in a **clockwise** direction five times.

RINSE the broom as quickly as possible into the PreservCyt® Solution vial by pushing the broom into the bottom of the vial 10 times, forcing the bristles apart. As a final step, swirl the broom vigorously to further release material. Discard the collection device.

TIGHTEN the cap so that the torque line on the cap passes the torque line on the vial.

RECORD the patient's name and ID number on the vial.

RECORD the patient information and medical history on the cytology requisition form

Courtesy of Cytyc Corporation

ThinPrep® Pap TestTM Collection Video Link

http://www.hosp.wisc.edu/uconnect/ThinPrepEducation/thinprepeducation.html

SPECIAL COLLECTION SITUATIONS

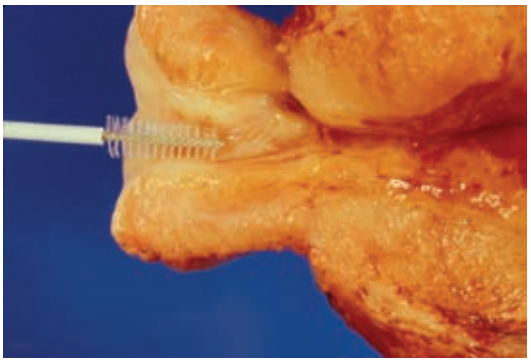
Post-Hysterectomy - It is important to note if the patient has had a supracervical hysterectomy. A cervical stump can be sampled in the same manner as an intact cervix. A woman without a cervical stump should have the Pap Test taken from the upper third of the vaginal vault.

Atrophic Vaginitis - It is important to visualize the cervix. If the cervix cannot be visualized or the Pap test is resulted as "unsatisfactory" treatment with an estrogen cream may be needed.

Pregnancy and Post-Partum - If a cervical brush is used, care must be taken to insert the brush only to the depth necessary. Gentle rotation should be used to obtain the sample. Post-Partum Paps should not be taken prior to six weeks post-partum due to increased false positive results, likely due to reparative changes in the cervix

ENDOCERVICAL CANAL CURETTAGE

What is an Endocervical Canal Curettage Pap (ECC Pap)?



Cells from the canal are gathered with a cytobrush to access if lesion is present in canal.

This is an acceptable alternative to the traditional curettage.

COLLECTION SUPPLIES

ThinPrep® Supplies

ThinPrep supplies can easily be ordered through central supply. The order numbers are as follows:

each packaged in groups of 25…

4002468	Collection broom
4002461	Collection broom (blue handle)
4002459	Plastic scraper
4002460	Brush
4002467	ThinPrep vials

WHEN TO REPEAT PAPS

To Repeat or Not to Repeat? That is the question!

Do repeat!

- For an ASCUS Pap in 6-12 months
- If unsatisfactory Pap Test result

Do Not repeat!

- When taking a biopsy – only an ECC brush should be done if necessary
- In less than 2 months – compromises cytology, cervix needs time to regenerate
- For endometrial cells – needs clinical exam and possible endometrial biopsy
- For missing endocervical component – before 6 months

Suboptimal times for Pap Test Collection

- **During menses and infection,** increased chance for unsatisfactory specimens.
- Within 48 hours of douching or use of vaginal medication, and vaginal contraceptives.

ThinPrep® Pap Test™ Collection Tips

- Always wipe away mucus from cervix before cell collection for a ThinPrep® Pap Test™ (however for a conventional smear do not wipe mucus away from cervix).
- Collect Pap specimen BEFORE the application of acetic acid or iodine solution.
- Do not use lubricant – doing so limits the amount of cellular material
- Identify the transformation zone, if visible and direct sampling efforts to encompass this area.
- **Tighten lids** of ThinPrep® vials before transport.
- **Always note the presence of an IUD,** which can simulate abnormal cellular changes, on the Pap Test requisition.
- Refrain from collecting Pap Tests **until after six weeks post-partum,** due to increased false positive results from reparative changes in the cervix.
- Lack of an endocervical component does **not** affect the adequacy of the specimen. Post-menopausal women may have no identifiable endocervical component, especially when there is marked epithelial atrophy.

THE BETHESDA SYSTEM

The Bethesda System (TBS) was the result of a workshop sponsored by the National Cancer Institute (NCI) in 1988 (with revision in 1991) to standardize terminology and reporting of cervical cytology. It has been widely adopted both in the United States and internationally.

In 2001, the NCI sponsored a third workshop-Bethesda 2001- to evaluate changes in the practice of cytopathology since TBS 1991, including methods, introduction of ancillary techniques/testing, and automation. The final recommendations of the group modify the existing Bethesda System-most notably as it deals with specimen adequacy and general categorization. Those revisions are summarized in the tables that follow.

Courtesy of CYTYC Corporation

TBS GENERAL CATEGORIZATION

TBS 1991	TBS 2001	CHANGE
Within Normal Limits (WNL)	Negative for Intraepithelial Lesions or Malignancy • Organisms • Other non-neoplastic findings	WNL is now named **Negative for Intraepithelial Lesions or Malignancy** and includes the previous category of BCC as a descriptor only.
Benign Cellular Changes (BCC) • Infection • Repair		BCC was eliminated as a diagnostic category (see above).
	Other Endometrial cells in a woman > 40 years old (specify if negative for SIL)	This category is new.

Courtesy of Cytyc Corporation

TBS GENERAL CATEGORIZATION

TBS 1991	TBS 2001	CHANGE
Satisfactory	**Satisfactory for Evaluation** (Describe presence or absence of endocervical transformation zone component and any other quality indicators, e.g., partially obscuring blood, inflammation, etc)	For liquid-based cytology, an adequate sample would have a minimum of 5,000 epithelial cells to be satisfactory. The presence of an epithelial cell abnormality automatically makes a specimen satisfactory-regardless of the number of epithelial cells.
Satisfactory but limited by (SBLB) • Lack of endocervical cells • Obscuring blood • Obscuring inflammation • Air-drying artifact		The category SBLB has been eliminated. The descriptors are to be used in a comment section, but not to determine adequacy.
Unsatisfactory • Obscuring blood • Obscuring inflammation • Air-drying artifact	**Unsatisfactory for evaluation** Specimen rejected/ not processed Specimen processed and examined, but unsatisfactory –too few cells, poor preservation, totally obscured by blood	The reason to refer to a specimen as unsatisfactory have been reduced to the reasons noted (left).

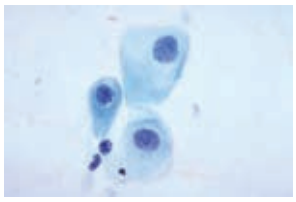
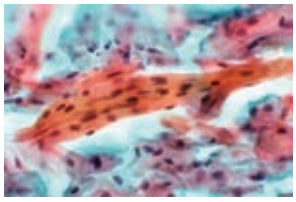
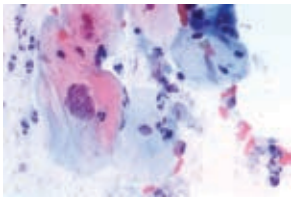
TBS 1991	TBS 2001	CHANGE
Epithelial Cell Abnormality: Squamous Cells • ASCUS (atypical squamous cells of undetermined significance) -favor reactive, favor dysplasia • LSIL • HSIL • Squamous cell carcinoma	**Epithelial Cell Abnormality: Squamous Cells** • Atypical squamous cells -of undetermined significance (ASC-US) -cannot excluded HSIL (ASC-H) • LSIL • HSIL • Squamous cell carcinoma	The multiple subcategories of ASCUS have been reduced to the two noted (ASC-US, ASC-H) with no other modifying statements.
Epithelial cell Abnormality: Glandular Cells • AGUS (atypical glandular cells of undetermined significance) -favor reactive, favor neoplasia • Adenocarcinoma	**Epithelial Cell Abnormality: Glandular Cells** [a] Atypical (NOS) -Endocervical cells, endometrial cells, glandular cells • Atypical (favor neoplastic)-endocervical cells, glandular cells • Endocervical adenocarcinoma in situ (AIS)	The subcategories of AGUS have been expanded to allow for a more descriptive diagnosis of glandular abnormalities. AIS is now a distinct entity.

Courtesy of Cytyc Corporation

ASCUS

Name: Atypical Squamous Cells of Undetermined Significance

How should it be triaged? Repeat Pap test in 6-12 months, reflex HPV testing

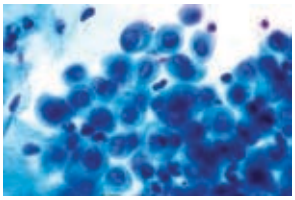
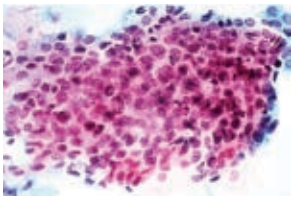
The different morphologic looks of ASCUS…

Nucleus of intermediate cell is 2-3 times normal, atypical parakeratosis, in addition to other unexplained atypias, smooth nuclear outlines

ASC-H

Name: Atypical Squamous Cells cannot rule out a High-grade neoplasm

How should it be triaged? Colposcopy, biopsy and ECC Pap Test or traditional ECC, NOT HPV reflex testing

The different morphologic looks of ASC-H…

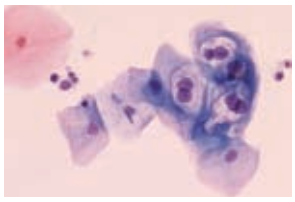
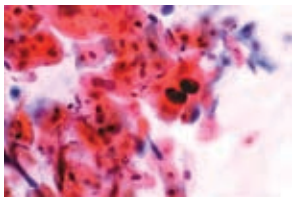
Nuclear to cytoplasmic ratio is increasing, nuclear hyperchromasia,

LSIL

Name: Low grade squamous intraepithelial lesion

What biopsy diagnosis correlates? CIN I

How should it be triaged? Colposcopy, biopsy, traditional ECC or ECC Pap Test. Patients ≤ 20 years of age should have repeat cytology only.

The different morphologic looks of LSIL…

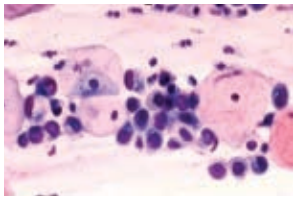
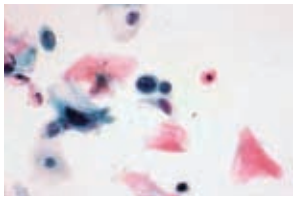
Enlarged, hyperchromatic nucleus, presence of koilocytes, binucleation, moderate variation in nuclear size and shape

HSIL

Name: High-Grade Squamous Intraepithelial Lesion

What biopsy correlates? CIN II, CIN III

How should it be triaged? Colposcopy, biopsy, traditional ECC or ECC Pap Test

The different morphologic looks of HSIL…

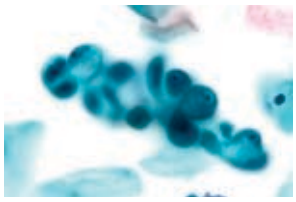
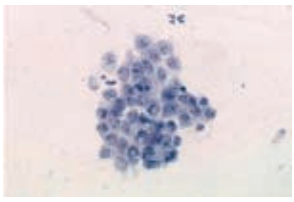
Small cells with hyperchromatic nuclei, increased nuclear to cytoplasmic ratio, immature cytoplasm, chromatin finely to coarsely granular, nucleoli are absent, irregular nuclear outlines

AGC

Name: Atypical Glandular Cells (endocervical, endometrial)

Clinical Significance: Possible glandular lesion that needs to be further investigated

How should it be triaged? Colposcopy, biopsy, ECC or ECC Pap Test, Endometrial sampling

The different morphologic looks of Atypical glandular cells…

Cell borders are ill-defined, nuclear atypia that exceeds obvious reactive or reparative changes

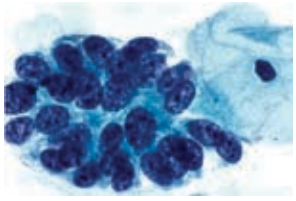
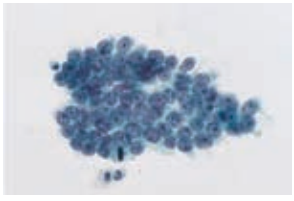
But lack unequivocal features of invasive adenocarcinoma.

AIS

Name: Adenocarcinoma In-Situ

Clinical Significance: A glandular lesion that needs to be further investigated

How should it be triaged? Colposcopy, ECC, biopsy

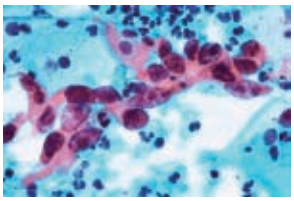
The different morphologic looks of AIS…

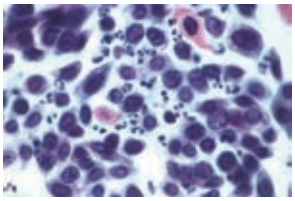
Abnormal cells occur in sheets, strips, and rosettes with nuclear crowding and overlap, ill-defined cell borders, variation in nuclear size and shape, mitotic figures may be seen.

SQCA

Name: Squamous Cell Carcinoma

How should it be triaged? Biopsy



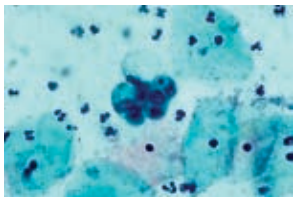


The different morphologic looks of SQCA…

Cells with increased ratios, irregular nuclear outlines, associated tumor diathesis consisting of necrotic debris and old blood are often present, cells occur singly, less commonly in aggregates, coarse chromatin, marked variation in cellular size and shape (caudate and spindle cells)

HISTORY

Important History to provide…



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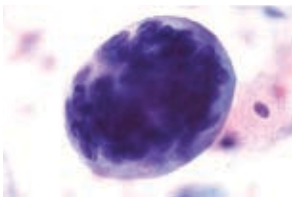


Pregnancy

Unsatisfactory Pap Tests are frustrating not only to the laboratory and clinic but also for the patient. Some things to keep in mind regarding Unsatisfactory Results

- Unsatisfactory Pap tests incorrectly designated as negative infer the absence of disease (SIL or malignancy) and may not prompt adequate follow-up
- Reporting numerically challenged Pap tests as "satisfactory" may have potential negative legal implications
- Unsatisfactory Pap tests are most often from high-risk patients, containing significantly more SIL/neoplasia on follow-up
- It is dangerous to label a specimen less than 5,000 cells satisfactory, since optimal detection rates of HSIL and LSIL are achieved when sample cellularity is greater than 20,000 cells

Going a Step Beyond

Because academic medical centers have many patients that are high risk, transient, or are referred because of previous abnormals the UWHC cytopathology laboratory has implemented three procedures. None of these procedures are a government issued requirement, but something that we have chosen to conduct for better patient care.

Two diagnosticians

All unsatisfactory Pap tests are reviewed and signed out by two cytotechnologists.

Glacial acetic acid procedure

Because blood is a leading reason for unsatisfactory Pap tests our laboratory monitors the color gradient of all ThinPrep® Pap vials. Any vial meeting a 2-3+ level of coloration is selected for additional processing. These specimens have a diluted acid added to help remove large amounts of blood. Therefore, fewer tests are called unsatisfactory.

Unsatisfactory Audit

Unsatisfactory rates for each clinician are recorded and monitored quarterly. At the end of each quarter, rates are sent to each clinician.

INSURANCE CARRIERS

In the past, the ThinPrep® Pap Test™ was reimbursed by only a few insurance companies, in most cases, for high-risk women only. Because of the success of this advanced test, insurance reimbursement rates have increased drastically. Currently low and high-risk women are able to receive the ThinPrep® Pap Test™. All National Insurance companies cover the test. Moreover, Medicare and Medicaid patients are also reimbursed. We are not aware of any insurance companies in the upper Midwest that do not reimburse for the ThinPrep® Pap Test™.

HPV

HPV

Member of family Papovaviridae

Double-strand DNA tumor viruses

Non-enveloped virion

More than 100 different types identified-highly specific for target epithelium

Anogenital HPV Types

Low-risk HPV types =6,11,42,43,44,54,61,70,72,81

(Low potential for progressing to cancer)

High-risk HPV types = 16,18,31,33,35,39,45,51,52,56,58,59,66,68,73,82

(High potential for progressing to cancer)

Munoz, et al, (2003) NEJM

HPV - EPIDEMIOLOGY

Epidemiology

- HPV DNA is associated with virtually all cervical cancers and their high-grade percursor lesions. (Bosch FX, et al, J Natl Cancer Inst.1995;87:796-802, Schiffman MH, et al, J Natl Cancer Inst. 1993;85:958-964)
- In a small minority of women exposed to high-risk HPV, the infection progresses to high-grade pre-invasive dysplastic lesions and cancer. (Walboomers JM, et al J Pathol 1999: 189:9-12)
- Acute HPV infection is common soon after initiation of sexual activity but most infections clear within 24 months
- The majority of cytologic abnormalities caused by HPV infection are low-grade cervical dysplasias and will resolve (Moscicki AB, et al. J Pediatr 1998: 132:277-84)

HPV - TESTING USE

When to Use HPV Testing

- A Pap Test result of Atypical Squamous Cells of Undetermined Significance (ASC-US)
- A Pap Test result of Atypical Squamous Cells cannot rule out a low-grade lesion (ASC-L)
- A recent negative Pap Test but a history of prior abnormal results
- As an adjunct to cytology screening in women ≥ 30 years old

When to Not Use HPV Testing

- An unsatisfactory Pap Test result
- A Pap test result of Atypical Glandular Cells (AGC)
- A Pap test result of Atypical Squamous Cells cannot rule out a High-grade lesion (ASC-H)
- A Pap test result of Low-grade Squamous Intraepithelial Lesion (LSIL)
- A Pap test result of High-grade Squamous Intraepithelial Lesion (HSIL)
- A Pap test result of Adenocarcinoma In-Situ (AIS) or invasive carcinoma
- Patient under the age of 20 with a low-grade squamous intraepithelial lesion (LGSIL)

How to order an HPV Test

- To order reflex HPV high risk testing from the Pap test specimen, choose either If Pap result is ASCUS or 'If Pap result is ASCUS or NEGATIVE' on the paper or electronic requisition.
- HPV Add-On testing: Paper Orders:
  - If HPV is ordered AFTER you receive the Pap test result, fax a copy of the Pap test report with the ordering physician/provider signature requesting HPV testing be done. Fax to the Cytology Lab @ 608-263-6453.
- HPV Add-On testing: Electronic Orders:
  - Select HPV add-on test options from HealthLink, UWHC test menu options.
- Orders for HPV testing off of the vial must be submitted to the laboratory within 3 weeks of the collection date. All Pap test vials must be discarded after three weeks of the collection date in compliance with the manufacturer's guidelines.

Frequently Asked Questions About HPV Testing

1. Why is testing for HPV low-risk types not offered?

With persistent infection, only high-risk types of HPV have been found to progress to cervical cancer. Therefore, the Cytopathology laboratory will conduct testing for high-risk HPV types only.

2. Can HPV testing be done if my patient is found to have a high-grade squamous intra-epithelial lesion?

No. HPV testing is to be used as a triage mechanism for patient care predominately in cases of equivocal Pap test results such as ASC-US. It is not a diagnostic tool. In patients over the age of 20, when a lesion is present, treatment is necessary regardless of the HPV result.

3. Where can I find additional information regarding HPV testing and the triage of patients?

The American Society for Colposcopy and Cervical Pathology www.asccp.org

Sherman ME, Shiffman M, Cox TJ. 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). Journal of the National Cancer Institute. 2002, 94: 102-107.

4. Can postmenopausal patients with negative HPV results discontinue Pap testing?

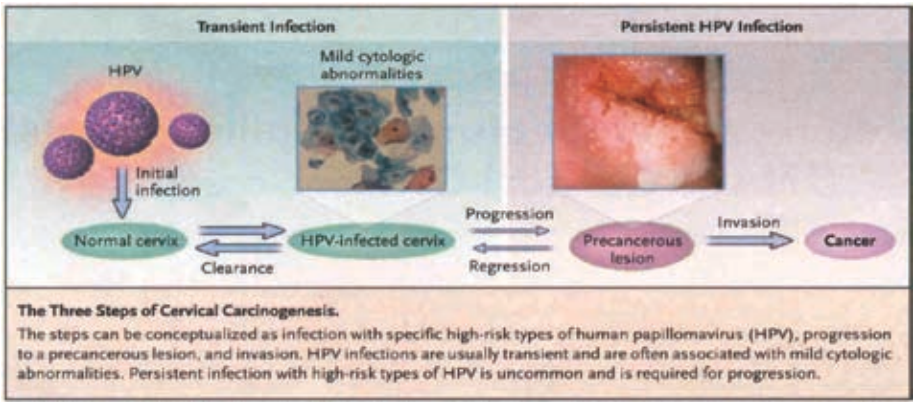
No. Postmenopausal patients that are sexually active still run the risk of contracting the HPV virus. In addition, patients could harbor the virus until immunocompromised due to illness or other health problems.

HPV Testing at UWHC

- As of 2008 the ThinPrep® Pap Test™ and the Third Wave Technologies Inc, Invader® HPV Test are combined. By using the liquid cytology sample collected for an initial ThinPrep® Pap Test™, you may request an HPV test using the Invader® assay.
- If you have requested HPV testing on the cytology requisition form and your Pap Test was ASC-US the laboratory will provide you with a statement on the report stating HPV testing has been sent. HPV testing normally takes an additional week. Results for HPV testing are found within the UW Health EMR (WISCR and Health Link) system.
- Knowing the HPV status of a patient with an ASC-US Pap smear can be of benefit in deciding the most appropriate management strategy for that patient. High-risk HPV DNA types have been shown to play a causal role in the development of cervical disease and cancer. Their presence in a patient with an ASC-US Pap smear indicates she is at increased risk for disease and would benefit from immediate colposcopy.

HPV INFECTION HISTORY

Natural History of HPV Infections



The Three Steps of Cervical Carcinogenesis.
The steps can be conceptualized as infection with specific high-risk types of human papillomavirus (HPV), progression to a precancerous lesion, and invasion. HPV infections are usually transient and are often associated with mild cytologic abnormalities. Persistent infection with high-risk types of HPV is uncommon and is required for progression.

Wright and Schiffman (2003) NEJM

Natural History of HPV

Development of CIN - summary:

- Almost one in four HPV DNA (+) women have an abnormal Pap within 5 years
- Development of CIN 2,3 is common - about 3-10% if high-rish HPV (+)
- Persistent infections are at great risk

Courtesy of Thomas C. Wright, Jr.

ASCCP ALGORITHMS

ASCCP Management Guidelines Link

http://www.asccp.org/pdfs/consensus/algorithms_cyto_07.pdf

Frequently asked questions

1. Should lubricants be used during Pap testing?

NCCLS Guidelines recommend that no lubricant be used during Pap testing. ACOG recommends that care be taken not to contaminate the specimen with lubricant because this may lead to an unsatisfactory result. Please refer to NCCLS Document: Papanicolaou Technique; Approved Guideline and ACOG Practice Bulletin, no. 45, August 2003. For physicians using plastic specula, or in instances where a lubricant must be used, take care not to contaminate the cervix or collection devices with lubricant. A tiny amount of lubricant may be used, just enough to sparingly coat the speculum with a gloved finger, avoiding the tip of the speculum.

2. Should patients post-hysterectomy for benign disease patients continue to have PapTests?

Although there is some controversy of Pap Tests in post-hysterectomy patients for benign disease our laboratory recommends the continuation of annual Pap testing.

3. What age should patients begin Pap Test screening?

Three years after the onset of sexual activity but no later than 21 years of age according to the America Cancer Society Guidelines.

FOR MORE DETAILS PLEASE VISIT...

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www.Bethesda2001.cancer.gov

The Bethesda Sytstem (nomenclature for reporting)

www.asccp.org

The American Society for Colposcopy and Cervical Pathology (includes information about consensus guidelines, Bethesda 2001, management of women with cytological abnormalities)

www.Cytyc.com

ThinPrep® Pap Test™ information