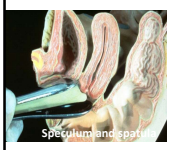
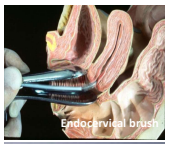
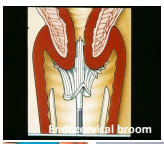
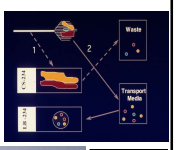
Pap Test



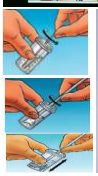
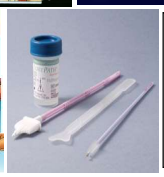






Both Pap and HPV tests addressed
21 years: begin screening
21-29 years: Pap test every 3 years
30-65 years: Cotest every 5 years (or Pap test every 3 years)
65 years: exit screening*

Saslow D., et al, Am J Clin Pathol. 2012; 516-42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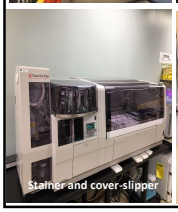

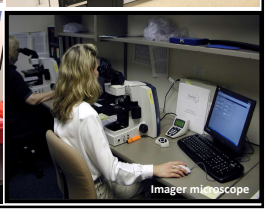

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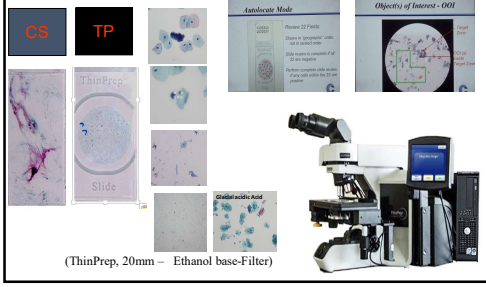
Hologic ThinPrep Imaging System



(ThinPrep, 20mm – Ethanol base-Filter)

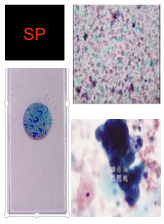
Selects 22 fields of view for a Cytotechnologist to review. Following review of these fields, the Cytotechnologist will either complete the diagnosis if no abnormalities are identified or review the entire slide if any abnormalities are identified.

4

PrepMate and PrepStain® System

5

BD FocalPoint GS Imaging system



(SurePath, 13mm- Methanol based- Density gradient).

BD FocalPoint™ GS Imaging System
Imaging technology identifies and ranks microscopic fields for cytotechnologist review
Designates slides for QC
10 FOV
If no abnormalities, FOV review only
If abnormal, Full Manual Review performed (FMR)

6

Cytology is a highly regulated subspecialty

- Wall Street Journal article in 1987 exposes lack of standards in laboratory practices
  - Suboptimal training
  - Long hours
  - Too many Paps per day.
  - Unregulated laboratories.
  - Payment of techs on a per-slide basis.
  - Bonuses for exceeding daily numbers.
  - Taking slides home to screen
- **CLIA 88 (10% rescreen):** Clinical Laboratories Improvement Amendments of 1988, Final Rule 1992

7

CLINICAL LABORATORY IMPROVEMENTS AMENDMENTS OF 1988 (CLIA 88)

Cytology is high complexity testing- mandated quality standards:

- Location of cytology testing.
- Methods of slide preparation and staining.
- Retention of records
- Personnel requirements and duties
- Established workload limits based on performance evaluations
- Hierarchical review of gynecological cases reactive or higher and all non-gynecological cases
- Quality control and quality assurance practices
- Statistical reports
- Proficiency testing

8

How often must laboratories be surveyed to maintain accreditation?

1. Every 2 years
2. Every 3 years
3. Every 5 years
4. Every 7 years
5. Every 10 years

9

Accreditation process

- On-site surveys of laboratories to ensure they meet the necessary requirements to be compliant with regulations
- Must be conducted **every 2 years** to maintain CLIA compliance
- CAP checklists: 09.17.2019

2019 CAP Cytopathologist Checklist revisions: Changes if Cytology does IH

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CLIA 88: Cytology-specific mandates

- **1. Personnel standards**
  - **Hierarchical review of slides**
- **2. Pre-analytics: Cytopreperatory**
  - **Staining**
- **3. Analytics**
  - **Rescreening**
  - **Workload limits**
- **4. Post Analytics**
  - **Cytology-histology correlation**
  - **Proficiency testing**
  - **Slide retention**
  - **Performance evaluations**

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1. Personnel standards:

Cytotechnologists
- Bachelors degree
- Must graduate from an accredited cytotechnology school, and pass board exam (ASCP)
- Screen and sign out negative GYN, except reactive/repair/inflammatory specimens (Herpes; Follicular cervicitis)
- Assist in FNA and ROSE

General supervisor
- CT with ≥ 3 years full time experience.
- Can participate in prospective Q/C rescreen and 5 year retrospective review for HSIL/malignancy.
- Assist with day-to-day running of lab

Technical supervisor/Medical director
- Licensed M.D. or D.O. with board certification in anatomic pathology (or AP/CP) from ABP
  - Sign out abnormal GYN including reactive/repair and all non-GYN cases
  - Evaluate and set workload limits/other medical director duties
- Technical supervisor/Medical director– BC/BE in Anatomical pathology
  - Confirms all non-negative gyn
  - Reviews/Reports all non-gyn including negatives

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Six Elements for Evaluating Competency

1. **Direct observations** of routine patient test performance, including patient preparation, if applicable, specimen handling, processing and testing
2. **Monitoring** the recording and reporting of test results
3. **Review** of intermediate test results or worksheets, quality control records, proficiency testing results, and preventive maintenance records
4. **Direct observations** of performance of instrument maintenance and function checks
5. **Assessment of test performance** through testing previously analyzed specimens, internal blind testing samples or external proficiency testing samples
6. Assessment of **problem solving skills**

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Hierarchical review of slides

- All non-GYN cases including anal cytology.
- Selected gyn cases are reviewed, either by a second cytotechnologist (who qualifies as a general supervisor), or by a cytopathologist.
- **These are Lab Dependent- No Specific CLIA Guidelines address this:** The general cytology supervisor reviews all cases screened by a new employee during the first two weeks of employment. Cytotechnologists with less than three years screening experience or PRN employees will require the general supervisor to "review 150 negative gynecological cases" for recent graduates or Board-eligible.
- NILM/positive HPV cases are included in the QC review by a second cytotechnologist

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Hierarchical review of slides: GYN cytology

Send to pathologist for review all cases specified below:

a. showing endometrial cells in a woman postmenopausal or patient age 45 or older
c. with cellular evidence of Herpes virus cytopathic effect, Actinomyces
d. with laboratory record of history of exposure to DES
e. showing significant reactive or reparative cellular changes
f. showing atypical cells of undetermined significance, or more severe changes
g. with a significant disagreement in diagnosis between the first and second cytotechnologist
h. about which there is some question
i. any described cervical abnormality stated in the clinical diagnosis area of requisition: bleeding cervix, erosion, ulcer, cervical mass, friable cervix (as examples).

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2. Preanalytic- Cytopreparatory: Cytopreparatory technician

- A. Stain Assessment (“done by qualified Cytotechnologist”)
- B. Cross-contamination
- C. Reagents/Labeling
- D. Equipment
- E. Temperature Control
- F. Numbering Cytology Samples
- G. Specimen Receipt Acceptance and Rejection Policy

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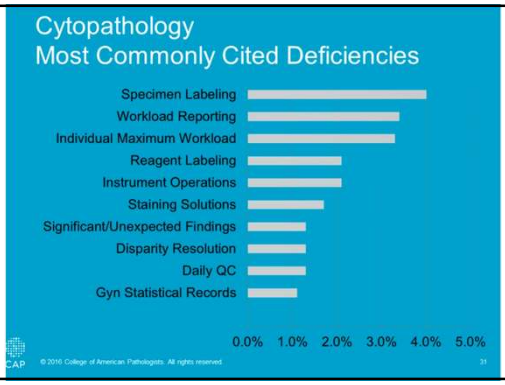

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3. Analytic

- **A. Procedure for Re-screen of negative GYN cases**
- **B. The QA rescreen percent**
- **C. Discrepant QC**
- D. Reclassified
- **E. Cytotechnologist workload**
- **F. Workload policy**
- G. Ten percent (10%) check on accessions.
- H. Internal check
- I. Safety reporting system (SERS)
- J. Consultation
- K. Hematology correlation
- L. Mandatory review

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3. Analytic:
A. Slide Rescreen: Mandated Quality Control Practices:

- Designed to reduce laboratory component of errors contributing to false negatives
- Mandatory **10% rescreening** of normal gynecologic specimens-Before the cases is reported.
  - Randomly selected cases (can include high risk patients)
  - "High-risk" patients (can added to 10%- no history- to an additional % that can total approximately of 18-25% negative cases reviewed)
- **5-year review**: If new HSIL or malignancy detected on Pap test, mandatory review of all NILM cases within **5** years prior.
  - If discrepancies found that affect current clinical management, must issue amended report and notify clinician

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E. Cytotechnologist workload

- Maximum 100 slides in an 8-hour workday (12.5 slides/hr)

GYN	Non-GYN
Imaged slide without manual review = 0.5 slide Imaged slide + manual review = 1.0 = 0.5 = 1.5 slides	Liquid-based (ThinPrep, cytospin, SurePath) = 1 slide Smear = 1 slide Cell block = 0.5 slide

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E. Cytotechnologist workload: Maximum workload limits

- Required manufacturers to revise their product labeling and send customer bulletins
- Published laboratorian safety tip

- The value of slides screened is as follows:
  - GYN manual review= 1 slide
  - GYN FOV only= .5 slide
  - GYN FOV + manual review= 1.5 slides
  - All non-GYN slides= 1 slide
  - Outside slides with a previous review/diagnosis=.5

- The exceptions to this are outside slide reviews that have been screened and resulted at another institution. The cases that contain IHC and cell block slides can be counted as .5 (many labs count them as 1 slide).
- **Upper limit is NOT for everyday productivity or a performance target**
- CLIA '88 requires *individual* maximum workload limits to be established by the technical supervisor

http://www.fda.gov/MedicalDevices/Safety/AlertsandNotices/TipsandArticlesonDeviceSafety/ucm220292.htm

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3. Analytic
F. Workload Policy

- Cytotechnologists are to screen all NON-GYN specimens assigned to them on a given day, and then to screen as many GYN slides as reasonably possible. The total number of slides screened is not to exceed 100 per 24 hour time period, or the individual workload limit determined for that cytotechnologist. Each Cytotechnologist has individually established workload limits that they are not to exceed. If a cytotechnologist screens less than 8 hours a day there is a formula to determine how many slides they can screen.
- The formula follows:

$$\frac{\text{number of hours spent screening X100}}{8} = \text{daily slides screened}$$

- i.e. 4 hours screened would mean the cytotechnologist could not exceed 50 slides for that day.

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E. Workload limits

- Workload limits evaluated and assigned every 6 months by Technical supervisor.
- Monthly workload tally sheets
  - Cases screened
  - Slides screened
  - Time spent screening, time spent not screening
- Workload includes slides, not cases
  - Gyn
  - Non-gyn
  - QC
  - Prof testing
  - Rescreen new hire

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4. Post Analytic

- **A. Adequacy Assessment Billing Audit**
- **B. Billing Report**
- C. Pending Urovision Report
- D. QA parameters
- **E. Cytology- Histology Correlation**
- F. Personnel Standards and Responsibilities
- G. Error Policy
- **H. Retrospective Review**
- **I. External Audits (Proficiency Testing/Educational Events)**
- **J. Final Reports**
- **K. Statistical Analysis**
- **L. Slide and Record Retention**
- **M. HR HPV results**

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4. Post Analytic
H. 5-year retrospective review of cases diagnosed as HSIL or higher

- CLIA mandates documented retrospective review of all negative gynecological specimens received in the preceding 5 years for patients with documented HSIL or above.
- If significant discrepancies are found, which affect current patient care, an amended report must be issued.
- Post Biopsy Review (PBR): Many laboratories perform 5 year review of negative Pap slides, triggered by a biopsy finding of HSIL, AIS, and above.

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4. Post Analytic:
I. External Audits- Proficiency testing (PT)

- Implemented in 2005, for GYN cytology only
- Each lab involved in GYN cytology must enroll in a CMS-approved PT program
- Each individual (pathologist and cytotechnologist) engaged in examination of *gynecologic* preparations must be *tested annually*.
- Lab director responsible for compliance

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4. Post Analytic:
I. External Audits-Proficiency testing (PT)

Each test set must contain at least one challenge representing each regulatory diagnostic category

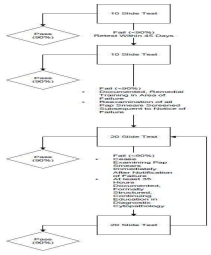
- A – Unsatisfactory
- B - Normal or benign
- C - Low grade SIL
- D - High grade SIL or cancer

Cytotechnologists:

Participant Diagnosis				
	A	B	C	D
Correct Diagnosis				
A	10	0	**5**	**5**
B	5	10	**5**	**5**
C	5	0	10	**10**
D	0	-5	**10**	10

Technical Supervisors:

Participant Diagnosis				
	A	B	C	D
Correct Diagnosis				
A	10	0	**0**	**0**
B	5	10	**0**	**0**
C	5	0	10	**5**
D	0	-5	**5**	10



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4. Post Analytic:
L. Slide and Record Retention

- Specific regulations regarding length of time that cytology laboratories must retain:
  - Accessioning records: 2 years
  - Test reports: 10 years
  - Gynecologic glass slides: 5 years
  - FNA glass slides: 10 years

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2019 CAP Cytopathologist Checklist revisions: CYP.06900 Slide Retention

- CAP changed Non Gyn slide retention for all case types to 10 years, cases diagnosed prior to December 31, 2014 are not subject to 10 year retention.
  - Prior checklist separated out FNA slides, which were kept for 10 years and all other non-GYN 5 years.
- Gyn slide retention remains at 5 years.

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Voices from the field: supporting the educational needs of cytotechnologists

- Results Research findings reveal CT education needs to align with emerging practice areas as reported in other Workgroup data collection efforts. The incorporation of new entry-level competencies in cytotechnology training programs prepares new CT graduates, but there is no standardized mechanism for formal, robust, and recognized ongoing education for other practicing CTs.

Friedlander MA et al. JASC 2018, 7, 250-260

30

A "Mid-level Pathology Practitioner" is a:

1) highly trained health professional;

2) who uses morphologic skill,

- understanding of neoplasia/disease

- ability to synthesize clinical and laboratory data

- to assist the pathologist

- in providing the highest quality diagnostic services.

"Mid-level Pathology Practitioners" expand the team-based model of the pathologist and cytotechnologist to include other, more advanced functions in the clinical laboratory arena.

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"Mid-level Pathology Practitioners"

- Requires changes in federal and state regulations
- Medical legal liability; insurance premiums for MLPP
- Loss of reimbursement for pathologists
- Hospital privileges would be required for FNA performance and independent non-gynecologic sign out

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Table 3 Perceived usefulness of education in various cytopathology-related practice areas.

Practice area category	Practice activity surveyed	Useful, %	Not useful, %
Cell Block	Cell block preparation*	81.5	18.5
	Cell block - Interpretation*	76.1	23.9
FNA Adequacy Assessment	Working as part of a clinical team with a cytopathologist and/or radiologist/clinician/surgeon*	78.6	24.4
	Determination of appropriate specimen triage for ancillary testing*	78.3	21.7
	Potential challenges and/or pitfalls in performing ROSE*	78.1	21.9
Immunocytochemistry	ICC/IHC - Interpretation*	75.4	24.6
	Selection of appropriate IHC stains*	73.2	26.8
	ICC/IHC - - Theory and validation protocols	70.9	29.1
	ICC/IHC - - Laboratory Process/Specimen processing	68.1	31.9
Procedurally FNA techniques	Triage of specimens (IHC/flow/molecular)*	74.8	25.2
	Endobronchial ultrasound–guided FNA*	74.4	25.6
	Endoscopic ultrasound–guided FNA*	74.3	25.7
	Clinico-radiologic-pathologic correlation*	68.9	31.1
	Transcutaneous FNA	59.8	40.2
Molecular Diagnostics	Molecular diagnostics - Theory & application to practice*	76.7	23.3
	Molecular diagnostics - Laboratory process/Specimen Processing	74.2	25.8
	New test integration	74.4	25.6
	Molecular diagnostics - QA/QC	73.2	26.8
	Interpretation (eg, ER/PR/HER2)	73.2	26.8

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Lab Management & Operations	Inspection readiness*	78.8	21.2
	Competencies/Competency Assessment*	78.3	21.7
	Regulatory compliance*	77.7	22.3
	QA non-GYN*	76.0	24.0
	Quality essentials, LEAN concepts, process improvement concepts; Quality Management*	74.3	25.7
	QA-GYN	73.1	26.9
	Document development and control	73.0	27.3
	Workload recording	69.7	30.3
	Personnel Management	65.4	34.5
	Performance/QI (LEAN, Six Sigma)	64.4	35.6
	Billing and coding	64.4	35.6
FISH/CISH	FISH/CISH - Morphologic assessment and	63.5	36.5
	FISH/CISH - New test integration	60.9	39.1
	FISH/CISH - Laboratory process	59.7	40.3
Circulating Tumor Cells	Circulating tumor cells - Interpretation	58.5	41.5
	Circulating tumor cells - Laboratory Process	59.3	40.7
Use of Computer-Assisted Devices	ThinPrep imaging system	55.2	44.8
	BD Focal Point GS imaging system	41.6	58.5
	GYN screening	49.3	50.7

*Topic incorporated as part of ACE conference agenda.

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Cytology-Histology Correlation

Fadi W. Abdul-Karim MD MEd

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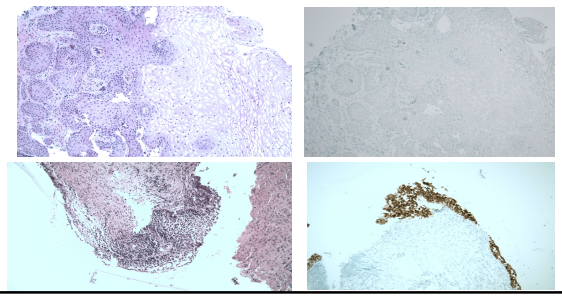
Pap HSIL: Biopsy results



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4. Post Analytic:
E. Gynecologic Cytologic- Histologic Correlation

The Clinical Laboratory Improvement Amendments of 1988 (CLIA) requires "Laboratory comparison of clinical information, when available, with cytology reports and comparison of all gynecologic cytology reports with a diagnosis of high-grade squamous intraepithelial lesion (HSIL), adenocarcinoma, or other malignant neoplasms with the histopathology report, if available in the laboratory (either on-site or in storage), and determination of the causes of any discrepancies." This requirement is generally referred to as cytologic-histologic correlation (CHC).

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4. Post Analytic:
E. Gynecologic Cytologic- Histologic Correlation

- **The practitioner may expect the biopsy to explain the abnormality on the smear, approximately 70%, 45% and 20% of cytologic interpretations of ASCUS, LSIL and HSIL, respectively, will not be verified on biopsy."**
  - Accuracy of cytology interpretation
  - Colposcopy evaluation
  - Clinical sampling error
  - Number of biopsies taken + Endocervical sample
  - Diagnostic imprecision.
  - Deeper levels obtained.
  - Consultation
  - Ancillary studies- IHC- P16

Crum, Cibas, Rose and Peters, Chapter 13 of Crum, Nucci, Lee, Diagnostic Gynecologic and Obstetric Pathology, 2011.

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Colposcopy

- Based on:
  - opacity
  - Margins
  - Contour
  - Vasculature
  - Staining reactions

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The Accuracy of Colposcopic Biopsy

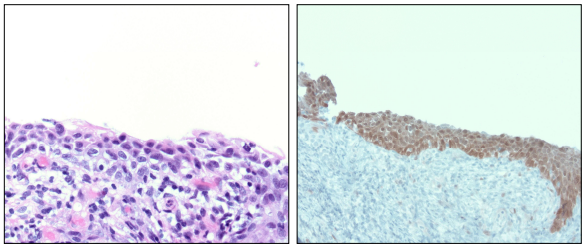
- "Colposcopy can easily determine the location and extent of 90% of cervical intraepithelial neoplasia (CIN) lesions." Morrow and Townsend's textbook
- **Colposcopic impression of HSIL identified only 56% of CIN2+ and the sensitivity for CIN 2+ of biopsy of colposcopically abnormal cervical epithelium is between 43.4% and 74.7% .**
- **The sensitivity of the first directed biopsy for CIN is around 52%.**

Apgar BS et al **Gynecologic procedures: colposcopy, treatments for cervical intraepithelial neoplasia and endometrial assessment.** Am Fam Physician. 2013 Jun 15;87(12):836-43

ACOG. Management of abnormal cervical cytology and histology. ACOG practice bulletin no. 99. Obstet Gynecol 2008;112:1419-44

40

Thin CIN: The inability of expert colposcopists to visualize some CIN 2/CIN 3 is associated with thinner epithelium.



Yang B **False-negative colposcopy is associated with thinner cervical intraepithelial neoplasia 2 and 3.** Gynecol Oncol. 2008 Jul;110(1):12-6.

41

Non-Correlating HSL and CIN1 or Less Biopsy sampling error

- 108 HSIL Paps and cervical biopsies < HSIL
- HSIL Pap test followed by cervical biopsy with or without subsequent cone/LEEP, there was a discordant cervical biopsy rate for HSIL of 43%.
- HSIL by Pap test followed up by cervical biopsy and subsequent cone/LEEP or repeat cervical biopsy, the proportion of women with negative colposcopic cervical biopsy and subsequent histology-proven HGCIN was 56%.
- **These figures justify sampling error as a valid cause of non-correlation in women with HSIL followed up by cervical biopsy alone.**

Hearp ML. **Validity of sampling error as a cause of noncorrelation.** Cancer. 2007 Oct 25;111(5):275-9.

42

Number of Cervical Biopsies: Sensitivity of Colposcopy

- **The sensitivity was significantly greater when the colposcopists took two or more biopsies instead of one, a pattern observed across all types of colposcopists.**
- Independent of the severity of the colposcopic impression, the frequency with which colposcopists took two or more biopsies instead of one varied (in descending order) from nurse practitioners to general gynecologists to gynecologic oncology fellows to gynecologic oncologists.

Gage JC et al. **Number of cervical biopsies and sensitivity of colposcopy.** Obstet Gynecol. 2006 Aug;108(2):264-72.

43

Multiple Lesion-Directed Biopsies

- 690 women: Up to four directed biopsies were taken from distinct acetowhite lesions and ranked by colposcopic impression. A nondirected biopsy of a normal-appearing area was added if fewer than four directed biopsies were taken.
- **Sensitivities for detecting HSIL increased from 60.6% from a single biopsy to 85.6% after two biopsies and to 95.6% after three biopsies.**
- **Taking additional biopsies when multiple lesions are present should become the standard practice of colposcopic biopsy.**

Wentzensen N **Multiple biopsies and detection of cervical cancer precursors at colposcopy.** J Clin Oncol. 2015 Jan 1;33(1):83-9

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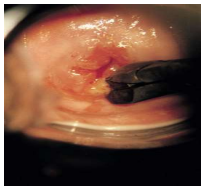
Pathology Requisition Form

- SPECIMEN SUBMITTED.
  A: Cervix, Biopsy 2:00
  B: Cervix, Biopsy 4:00
  C: Cervix, Biopsy 7:00
  D: Cervix, Biopsy 10:00
  E: Endocervical, Curettings
- SPECIMEN SUBMITTED:
  A: Cervix, Biopsy 3,6,9,12
  B: Endocervical, Curettings



Cervical Biopsy, Endocervical Curettage, and Cervical Biopsy During Pregnancy. Baggish, Michael S., Atlas of Pelvic Anatomy and Gynecologic Surgery, Chapter 45, 499-504. Elsevier

45

ATHENA trial: Random Biopsy and Negative Colposcopy: Genotyping

- ATHENA trial: 47,000 women with cytology and H-HPV genotyping. Colposcopy was performed in all women with abnormal cytology or positive HPV. A single random biopsy was taken at the SCJ if colposcopy was adequate/no lesion.
- The random biopsy diagnosed 20.9% and 18.9% of the total CIN2+ or CIN3+ 3, respectively. This additional disease was detected in both HPV 16 or 18+ and for 12 other high-risk HPV+ women.
- **Supports performing a random biopsy in women undergoing colposcopy without visible lesions, particularly in those positive for HPV 16 or 18 (AITS ? Larger lesions).**

Huh WK **Relevance of random biopsy at the transformation zone when colposcopy is negative.** Obstet Gynecol. 2014 Oct;124(4):670-6.

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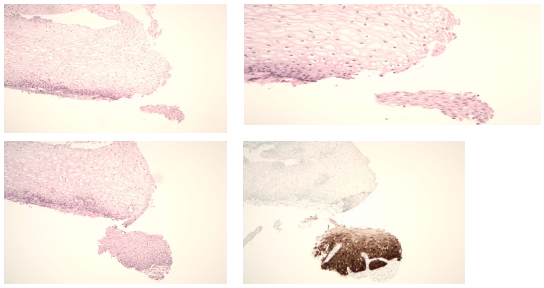
Deeper Levels: How many

- 600 consecutive biopsies from 404 patients were reviewed.
- If sectioning were limited to 3 levels, 17.5% (105/600) of all dysplastic lesions would have been missed, including 19.6% (100/511) of CIN 1 and 5.6% (5/89) of CIN 2-3.
- **Using our clinical efficacy standard, when no pathologic findings are initially identified in a colposcopic-directed biopsy, at least 5 levels (a priori or in recuts) are required to ensure a 100% diagnostic accuracy for CIN 2-3**.

adercP, Rodriguez R. **Squamous dysplasia of the uterine cervix: tissue sampling-related diagnostic considerations in 600 consecutive biopsies.** Int J Gynecol Pathol. 2007 Oct;26(4):469-74.

48

Deeper Levels



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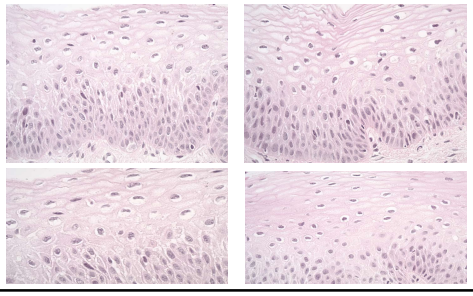
ALTS: Inter-observer Viability of CIN1

- **An interpretation of CIN 1 by the CC was corroborated by the QC group in only 42.6% of 887 biopsies.**
- **Equal proportion of originally diagnosed CIN 1 biopsies (41.0%) were interpreted as negative by the QC group.**
- CIN 1 diagnosed on a colposcopically directed biopsy who undergo a LEEP have identified CIN 2,3 in 23–55% of the excised specimens.
- **The poor reproducibility of the histologic diagnosis of CIN 1, as well as the uncertain biological potential of lesions that are classified based on their histologic appearance as CIN 1, makes management of these women problematic.**

Stoler MH. Inter-observer reproducibility of cervical cytologic and histologic interpretations: realistic estimates from the ASCUS-LSIL Triage Study. JAMA. 1994 Nov 2;272(17):1344-8.
Wright TC Jr. 2006 Consensus guidelines for the management of women with cervical intraepithelial neoplasia. J Low Genit Tract Dis. 2007 Oct;11(4):223-39.

50

LSIL ?



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The LAST Project

The Lower Anogenital Squamous Terminology Standardization Project for HPV-Associated Lesions: Background and Consensus Recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology.

Darragh TM et al

•Archives of Pathology and Laboratory Medicine:. October 2012 – Volume 135 – p 1266-1297.
•Journal of Lower Genital Tract Diseases. July 2012 – Volume 16 – p 205-242

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p16 Immunohistochemistry

Negative p16

- Cytoplasmic only staining
- Focal or patchy staining
- Discontinuous staining of basal layer
- Staining of upper layers but nor basal layer

Positive stain

- Diffuse (>80%) strong block positive nuclear or nuclear and cytoplasmic staining of basal layer and extending up at least 1/3 of epithelial thickness:
  - Correlates with presence of HR-HPV and diagnosis of dysplasia
  - Grading of dysplasia MUST be based on histology

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P16 not to be used

- A negative p16 is of greater significance than positive.
- Do not use to separate CIN1/ 2 or 2/ 3.
- HSIL HPV negative probably metaplasia but can use CIN undetermined or atypical squamous epithelium cannot exclude SIL
- P16 mainly needed for young women CIN2 to assure diagnosis so not over treat

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False positive P16

- Squamous morules from endometrium (are CDX2 +)
- Papillary syncytial metaplasia of endometrium.
- Small cell carcinomas of cervix
- Leiomyosarcoma
- Glandular lesions: Need significant tissue.
  - Focal staining in tubal metaplasia.
  - Focal strong in Endometrial carcinoma
  - Extensive staining in serous carcinoma.
  - Minimal deviation ca and mesonephric ca are negative.
  - Cervical endometriosis can have one gland positive but next to it a negative endometrial gland.

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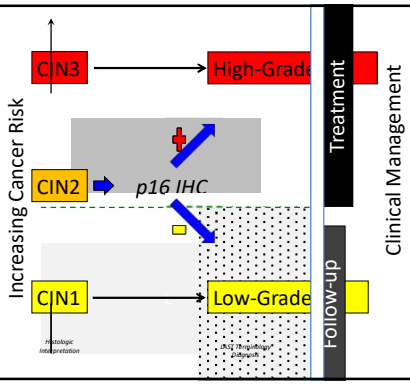

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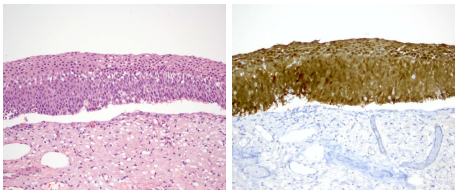
CIN2: P16



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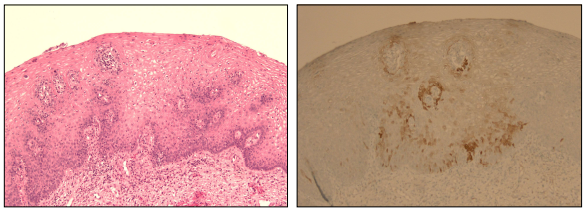
LSIL and P16



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Risk stratification by p16 immunostaining of CIN1 biopsies: a retrospective study of patients from the quadrivalent HPV vaccine trials.

- 524 patients with CIN1: 63 (12.0%) CIN2/3 on follow-up.
- **p16 IHC does not risk stratify CIN1 patients in a manner that would alter recommended management for CIN1. This reinforces the LAST recommendations that p16 should only be used selectively for problematic scenarios, such as CIN2 because of its inherent lack of reproducibility, cases in which one is struggling between CIN1 and CIN2, and benign mimics of CIN3.**

Mills AM, et al. Am J Surg Pathol. 2015.

59

p16 staining has limited value in predicting the outcome of histological low-grade squamous intraepithelial lesions of the cervix.

- LSIL/CIN1 at biopsy (n=507). p16 was positive in 245/507 patients (48%) and in 210/416 patients (50%) with confirmed LSIL/CIN1 at re-evaluation. Although positive p16 immunostaining was associated with risk of HSIL outcome in the multivariate analysis, in the overall group of patients with LSIL/CIN1, this association was not verified in the subset of patients with confirmed LSIL/CIN1 after re-evaluation.
- **LSIL/CIN1 lesions p16 should be limited to equivocal cases in which HSIL/CIN2 is included in the differential diagnosis since it has low value in clinical practice as a marker of progression of LSIL/CIN1.**

- Sagasta A et al Mod Pathol. 2016 Jan;29(1):51-9

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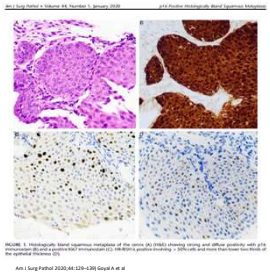
p16 Positive Histologically Bland Squamous Metaplasia of the Cervix



p16 positivity in squamous metaplasia of cervix is associated with the presence of transcriptionally active high-risk HPV even when there are no clear morphologic features of dysplasia. These lesions are ? early SILs or SILs that are not yet morphologically evident, most of which arise from SCJ.

"p16 positive squamous metaplasia." An explanatory note should be included stating that a focus of squamous metaplasia is identified in the sample that exhibits strong and diffuse immunoreactivity with p16 but does not fulfill the histologic criteria for SIL and that close clinical follow-up is recommended.

Am J Surg Pathol 2020;44:120–130| Goyal A et al

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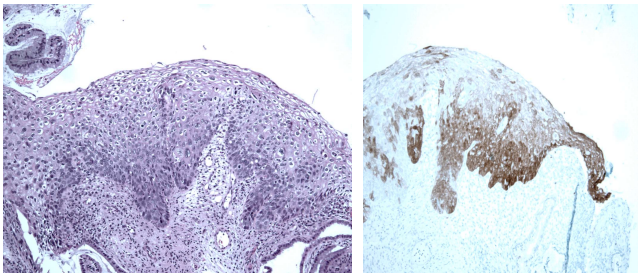
HSIL (CIN 2)



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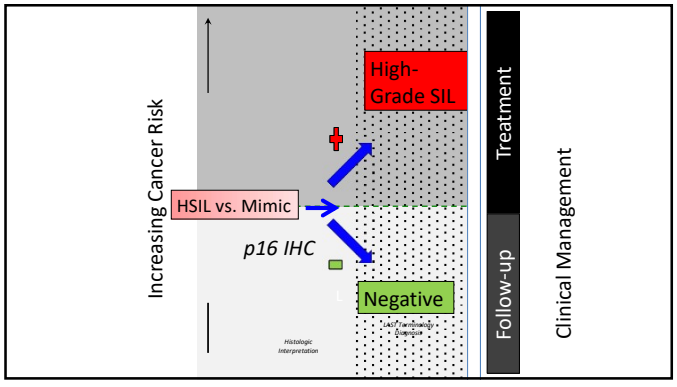

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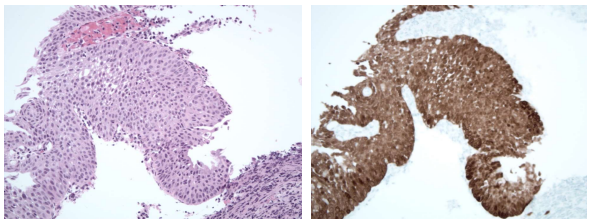
Immature squamous metaplasia vs. HSIL



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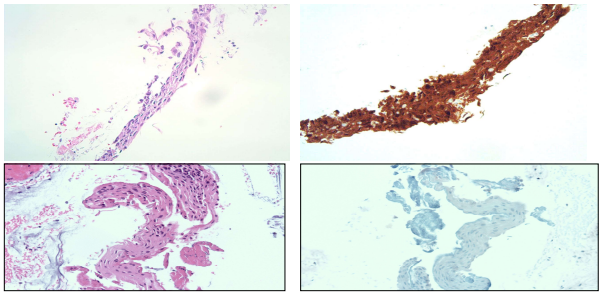
Atrophy vs CIN3



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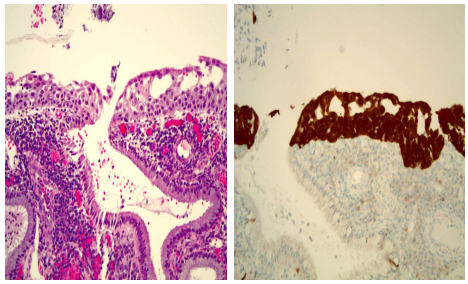
Atypical repair vs. HSIL



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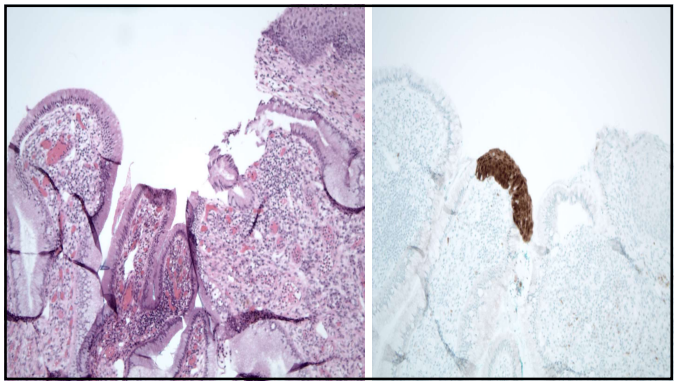
P16: Recommendation No. 4a

- *Special Circumstance*.—**p16 IHC is recommended as an adjunct to morphologic assessment for biopsy specimens interpreted as ≤–IN 1 that are at high risk for missed high-grade disease, which is defined as a prior cytologic interpretation of HSIL, ASC-H, ASC-US/HPV-16 +, or AGC (NOS).**
- **Any identified p16-positive area must meet** H&E **morphologic criteria for a high-grade lesion to be reinterpreted as such**.

67



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Examination of Sources of Diagnostic Error Leading to Cervical Cone Biopsies with No Evidence of Dysplasia

- 53 cone biopsies initially reported as negative for dysplasia or malignancy (17% of all cone biopsy specimens).
- Each negative cone biopsy specimen was examined with at least 3 deeper levels. If dysplasia not identified on deeper levels, p16 stain was performed on the most atypical level.
- Additional review by 3 pathologists for consensus diagnosis
  - 14 cases (26.4%) showed dysplasia to be present by at least 1 of the additional modalities (6 LSIL, 5 HSIL, 3 SIL)
  - 4 cases (7.5%) were identified by **additional level** sections (two-dimensional sampling of a 3-dimentional specimen)
  - 7 cases (13.2%) were identified by additional levels and **p16**
  - 3 cases (5.7%) were found by **consensus review**

Cartigg A, et al. *Am J Clin Pathol* 2013; 139:422-427.

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Examination of Sources of Diagnostic Error Leading to Cervical Cone Biopsies with No Evidence of Dysplasia

- Remaining 39 cases that remained negative with additional workup:
  - 15 cases (28.3%) were attributed to over interpretations on pre-surgical specimens.
  - 24 patients had confirmed HSIL on pre-surgical specimens but negative cone biopsy specimens, and 6 of 20 of these patients (11.3% of the total) with follow-up had confirmed dysplasia or carcinoma on subsequent specimens.
  - Therefore, the overall false-negative rate for cone biopsy specimens, when the fourth category of under-sampling was added, was 21%, a hardly insignificant proportion.

Carrigg A, et al. *Am J Clin Pathol* 2013, 139:422-427.

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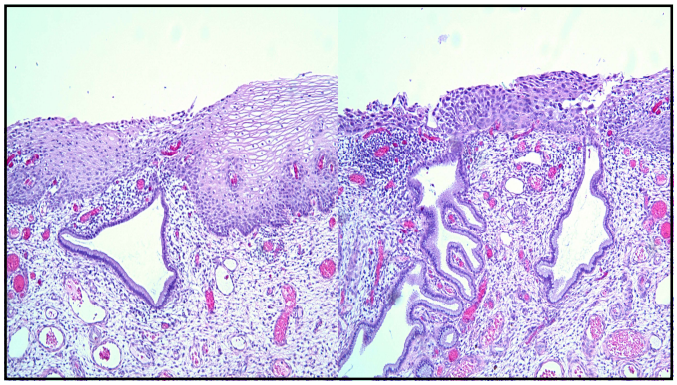

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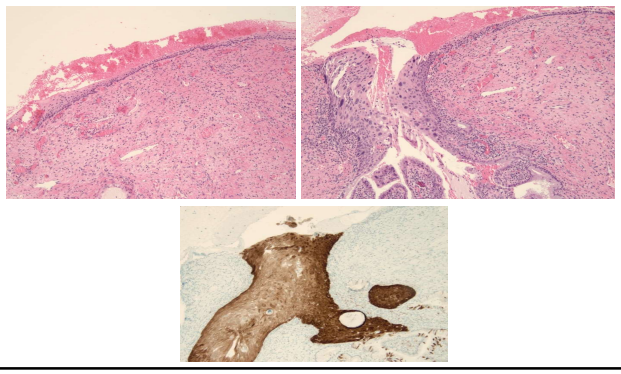

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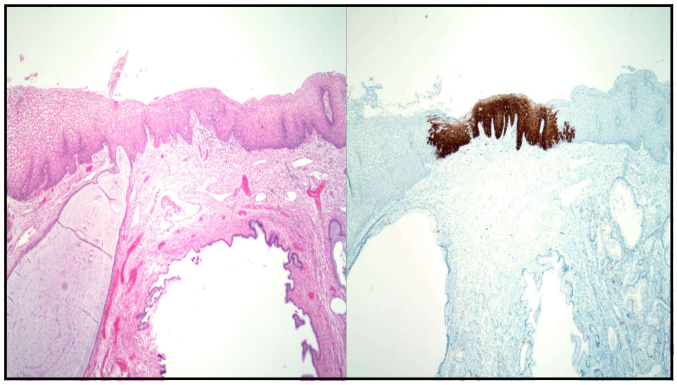

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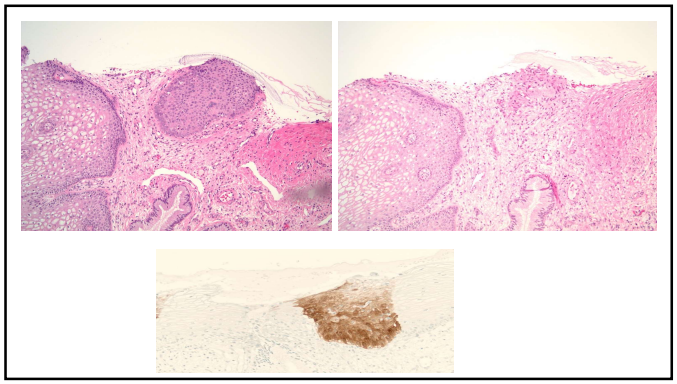

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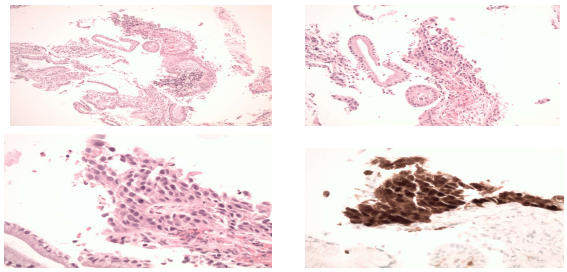
Cytologic- Histologic Correlation: Enhancing Cytologic-Histologic Correlation in Surgical Pathology: Prior Pap HSIL- Initial interpretation Negative-Deeper levels and P16 stain



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Sample Report Prior H- Pap and CIN1 or Less on Biopsy

- 1. Endocervical, curettings (A):

  - Fragments of endocervical epithelium with no significant pathologic findings.

- 2. Ectocervix, biopsy at 4 o'clock (B):

  Low-grade squamous intraepithelial lesion (CIN I). See comment.

Comment:

Deeper levels were obtained. Immunohistochemical stain for P16, done for prior HSIL shows focal patchy staining supporting the above interpretation. The prior Pap test C16-33880 was reviewed and the interpretation of HSIL is reaffirmed (Staff consultant: Dr. …….).

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Cytologic- Histologic Correlation: Enhancing Cytologic-Histologic Correlation in Surgical Pathology

- Specificity of cytologic interpretation
- Inter-Observer Reproducibility of Cervical Cytology
- Accuracy of Histopathologic diagnosis of SIL
- HPV testing and Genotyping
- Quality of Cytopathology and Histology laboratory
- Sampling issues and Obtaining deeper levels from tissue block
- Immunohistochemistry for p16 for HSIL diagnosis
- Consensus pathology reviewing with colleagues

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