THE BETHESDA SYSTEM FOR CERVICAL CYTOLOGY – UPDATE AND BEYOND

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Objectives

• Discuss the impact of the Cervical Cytology Bethesda System on Standardized Reporting Terminology and Management Guidelines
• Special emphasis on glandular abnormalities
TBS - Historical perspective

- Terminology principles:
  - Communicate clinically relevant information from the lab to the patient’s health care provider
  - Uniform and reproducible across different pathologists and labs
  - Flexible to be adapted to a wide variety of lab settings and geographic situations
  - Reflect the most current understanding of the disease process

Why a Third edition?

- Changes in practice of GYN cytology
  - Further insights in HPV biology
    - Primary HPV screening with Pap as diagnostic triage
    - New screening and management guidelines
    - Changes in histopathology terminology
    - Implementation of HPV vaccination
  - New data and technology
    - Additional experience with LBP
    - Endometrial cells, anal cytology, biomarkers, automation, risk assessment
    - Important for standardization of terminology for trials and research

HPV vaccines available in the U.S.

- Gardasil® (Merck) prevents infection of four strains of HPV – 6, 11, 16, and 18 – and was approved by the FDA in 2006 for use in males and females ages 9-26.
- In December 2014, Gardasil 9 was approved by the FDA. Protects against 9 strains of HPV: the four strains approved in the previous Gardasil vaccine, as well as 31, 33, 45, 52, and 58.
- Cervarix® (GlaxoSmithKline’s) was approved by the FDA in 2009 and protects against HPV strains 16 and 18. Cervarix can only be administered to females and has been approved for females ages 10-25.
HPV Vaccine Uptake

• Approximately 39.7% adolescent girls aged 13-17 received all three doses of the vaccine in 2014 up from 37.6% in 2013.
• Hispanics and girls whose families live below the poverty line were more likely to receive three doses compared to whites and girls whose families live above the poverty line.
• HPV vaccination rates among teen boys are much lower than for girls (21.6% in 2014), but has increased from 13.4% in 2013
• Prevalence of HPV in girls 14-19 yrs. has decreased significantly since introduction of the vaccine from 11.5% in 2003-2006 to 5.1% in 2007-2010

Sexually Transmitted Infection Vaccine Development

• HSV vaccine candidates are furthest along in the pathway, with several candidates in Phase I and II trials.
• Genital chlamydia vaccine: first Phase I human clinical trials started in 2016
• Gonorrhea and syphilis vaccine development is in earlier stages
• Vaccine development for trichomoniasis will require better epidemiologic, natural history, and basic science data

Sami L. Gottlieb; Christine Johnston. Curr Opin Infect Dis. 2017;30(1):77-86
Expected Impact of Removing HPV16/18 on Incidence of Abnormal Pap

<table>
<thead>
<tr>
<th>PAP CATEGORIES</th>
<th>MEAN INCIDENCE %</th>
<th>HPV %</th>
<th>HPV 16/18 Vaccine</th>
<th>Impact HPV 16/18</th>
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<tbody>
<tr>
<td>Normal</td>
<td>94</td>
<td>10</td>
<td>2.5</td>
<td>+</td>
</tr>
<tr>
<td>ASC-US</td>
<td>3</td>
<td>50</td>
<td>~20</td>
<td>++</td>
</tr>
<tr>
<td>LSIL</td>
<td>2.5</td>
<td>83</td>
<td>~25</td>
<td>++</td>
</tr>
<tr>
<td>HSIL</td>
<td>0.5</td>
<td>98</td>
<td>53</td>
<td>+++</td>
</tr>
<tr>
<td>Cancer</td>
<td>0.001</td>
<td>100</td>
<td>70</td>
<td>++++</td>
</tr>
</tbody>
</table>

Total reduction abnormal cytology: 15-20%
Reduce overall incidence of HSIL ~50% and cancer by ~70%
Why a Third edition?

• Updates and refinement of morphologic criteria
• Minimal changes in terminology
• Additional guidance and clarification
  – Specimen adequacy: lack of T-zone component
  – LSIL + possible HSIL: how to report?
  – Benign endometrial cells
    • Significance on Pap
    • Reporting issues

The Bethesda System

• Specimen type
• Specimen adequacy
  – Satisfactory or Unsatisfactory (specify reason)
• General categorization (optional)
  – NILM
  – Other (EMCs)
  – Epithelial cell abnormality (squamous or glandular)
• Interpretation

The Bethesda System

• Negative for intraepithelial lesion or malignancy
  – Including reactive changes and organisms
• Atypical squamous cells
  – ASC-US
  – ASC-H
• LSIL
• HSIL
• Atypical glandular cells
  – NOS: endocervical, endometrial, glandular
  – Favor neoplastic: endocervical, glandular
  – AIS
• Cancer: squamous, glandular, other
The Bethesda System

- Adjunctive testing
  - Brief description of test method and result
- Computer-assisted interpretation of cervical cytology
  - Specify device and result
- Educational notes and comments (optional)
  - Concise and consistent with published guidelines

Specimen adequacy

- Golden rule: Any specimen with abnormal cells is by definition satisfactory for evaluation
- Presence of benign endometrial cells at any age does not make an unsatisfactory specimen satisfactory

Specimen adequacy

- Cellularity
  - LBP: minimum 5,000 squamous or sq. met cells
    - To estimate count average number of cells in 10 micr. fields at 40x
  - Conventional: Estimate minimum of 8,000-12,000 squamous cells
- Transformation zone
  - Not necessary for adequate specimen
  - Report presence or absence of T-zone component
    - Quality indicator for clinical care provider = sampling quality
  - 10 well preserved endocervical or squamous metaplastic cells, singly or in clusters
- Atrophy: difficult to differentiate atrophic T-zone from parabasal cells
  - TBS: “No identifiable T-zone component in an atrophic pattern sample”
Quality indicator: No T-zone on Pap

- No T-zone on 10-20 % of Paps
- More frequent in pregnant and older women
- Meta-analysis on negative Paps
  - Regardless of +/- T-zone
  - Good specificity and NPV
- HPV test result independent of T-zone sampling

Elumir-Tanner L. CMAJ 2011;183:563-8
Zhao C. Gynecol Oncol 2007; 107:231-5

Non-neoplastic findings

- Squamous metaplasia
  - Difficult area when nuclear enlargement without nuclear abnormalities → avoid overinterpretation
- N/c ratio of less than 50%
- Smooth nuclear contours
- Even distribution of chromatin
- Higher N/C ratio + hyperchromasia + nuclear contour irregularities (notching or grooving) → consider ASC-H of HSIL
Non-neoplastic findings

• Tubal metaplasia
  – Metaplastic phenomenon: endocervical glands replaced by epithelium similar to normal fallopian tube
  – Frequently found in LUS
  – Nuclei enlarged, pleomorphic and hyperchromatic
  – Evenly distributed chromatin
  – N/c ratio high
  – Discrete cytoplasmic vacuoles or goblet cells
  – Presence of cilia and/or terminal bars
  – Most common pitfall for endocervical atypia or AIS

TBS Atlas: https://bethesda.soc.wisc.edu/introduction.htm
Pitfall

Tubal Metaplasia

AIS

Non-neoplastic findings

• Pregnancy-related cellular changes
  – Frequent pitfall for pre-neoplastic abnormalities
  – Pregnancy = incomplete maturation with intermediate-cell dominant pattern (navicular cells)
  – Decidua: From hormonally stimulated endocervical and endometrial stroma
    • Abundant granular or finely vacuolated cytoplasm
    • Large nuclei, lobulated or multinucleated
    • Fine chromatin pattern, normo- to hyperchromatic
    • Smooth nuclear membranes
    • Prominent nucleoli
Endometrial cells:
The how and when of reporting

- In PM women, exfoliated endometrial cells are considered abnormal and raise the possibility of endometrial neoplasia
- Menopausal status often unclear or unknown to the lab
- Median age of final menstrual period is 51 yrs., but variation is large
- TBS 1988: Report benign EMs in PM
- TBS 2001: Report in all women >40 years
  - Clinician to determine if further evaluation is needed
  - Led to unnecessary endometrial samplings

Consequence of TBS 2001

- Increased reporting of benign-appearing EMs
  - 0.17% to 0.49% of Pap (3X)
  - Decreased predictive value for hyperplasia and cancer

Risk associated with benign-appearing EMs on Pap

<table>
<thead>
<tr>
<th></th>
<th>Pre-2001</th>
<th>Post-2001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperplasia</td>
<td>12%</td>
<td>2%</td>
</tr>
<tr>
<td>Cancer</td>
<td>6%</td>
<td>1%</td>
</tr>
</tbody>
</table>
Summary of change in TBS 2014

• Endometrial cells are reported in women ages 45 and greater
  – To increase PPV and reduce unnecessary endometrial biopsies
• NILM / Endometrial cells are present in a woman >45 years of age (or) Endometrial cells correlate with the menstrual history provided
• The educational note specifies endometrial evaluation only in PM women
  – Note: Endometrial cells in women 45 years and older may be associated with benign endometrium, hormonal alterations and less commonly, endometrial or uterine abnormalities. Endometrial evaluation is recommended in postmenopausal women.

Which cells should be reported?

Endometrial cells
Endometrial stromal cells

Squamous epithelial lesions

Squamous epithelial lesions
LSIL + ASC-H

- LSIL with some cells suggestive of HSIL
- Modified TBS reporting:
  - LSIL, cannot exclude HSIL
  - LSIL-H
- Risk for HSIL on biopsy intermediate between:
  - LSIL and HSIL on cytology
  - Risk similar to ASC-H
Should LSIL-H Be a Distinct Cytology Category?
A Study on the Frequency and Distribution of 40 Human Papillomavirus Genotypes in 809 Women
Helain Zhou, MD, PhD, Mary R. Schwartz, MD; Gianna Coituy, MD; Danielle Smith, CT;
Dina H. Myers, MD, and Jiffie Bai, MD

ABSTRACT:
The WTEC study objective was to determine the frequency and distribution of 40 human papillomavirus genotypes, including low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), and adenocarcinoma in situ (AIS), in 809 women with cervical cytology. The study included 809 women who had undergone cervical cytology, including LSIL, HSIL, AIS, or both, and who had undergone HPV testing. The study was conducted over a period of 12 months. The study results showed that the frequency of LSIL was highest in women aged 20 to 29 years, while the frequency of HSIL was highest in women aged 30 to 39 years. The study also showed that the frequency of AIS was highest in women aged 40 to 49 years. The study results have implications for the management of cervical cytology in women.
LSIL-H

Squamous epithelial lesions
LSIL + ASC-H

• No new category
  – TBS 2014 keeps a 2-tier system (LSIL/HSIL)
  – Biology does not support an intermediate category
  – Expected poor reproducibility and overutilization
  – May lead to management confusion

• Report as ASC-H + LSIL
  – Should be the minority of cases

LSIL + ASC-H
Glandular abnormalities

• Cervical cytology is primarily a screening test for SIL and SCC
• Sensitivity for detection of glandular lesions limited to both sampling and interpretation
• AIS is the glandular counterpart of HSIL and precursor to invasive adenocarcinoma
• Similar HPV types in most but not all EAca and AIS
  – Proportion of EAca associated with HPV18 is larger than in SCC
• AGC defines an increased level of risk rather than a specific neoplastic precursor

Epithelial Abnormalities: Glandular

• Atypical
  – Endocervical cells (NOS)
  – Endometrial cells (NOS)
  – Glandular cells (NOS)
• Atypical
  – Endocervical cells, favor neoplastic
  – Glandular cells, favor neoplastic
• Endocervical adenocarcinoma in situ
• Adenocarcinoma
  – Endocervical
  – Endometrial
  – Extrachrine
  – Not otherwise specified

Epithelial Abnormalities: Glandular

• AGUS not utilized; to distinguish from ASC-US
• AGC needs to specify site of origin (EC or EM) if possible, since management differs by site
• Atypical EC cells and AGC may be subclassified as “favor neoplastic”
  – Avoid “favor reactive” → misleading
  – If not further qualified, use NOS
• Atypical EM cells are NOT qualified as to NOS or favor neoplastic due to difficulty in subclassification
Atypical Endocervical Cells

- **NOS**: EC that display nuclear atypia that exceeds obvious reactive or reparative changes but lack unequivocal features of AIS or IACa
- **Favor neoplastic**: Cell morphology (# & quality) falls short of an interpretation of AIS or IACa

AGC

- POSSIBLY REACTIVE
- SECONDARY TO RADIATION

AGC

- POSSIBLE TUBAL METAPLASIA

TBS Atlas [https://bethesda.uw.washington.edu/introduction.htm]
**AGC, NOS vs. AGC, favor neoplastic**

<table>
<thead>
<tr>
<th>Feature</th>
<th>AGC, NOS</th>
<th>AGC, favor neoplastic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Honeycomb, sheets, with minimal overlap</td>
<td>Crowding, overlapping, pseudostratification</td>
<td>Crowding, overlapping, pseudostratification</td>
</tr>
<tr>
<td>Round or oval nuclei, smooth contours</td>
<td>Nuclear enlargement (3-5X)</td>
<td>Rare rosettes &amp; feathering</td>
</tr>
<tr>
<td>Minimal anisonucleosis</td>
<td>Mild anisonucleosis</td>
<td>Nuclei enlarged and elongated</td>
</tr>
<tr>
<td>Multinucleation</td>
<td>Mild hyperchromasia</td>
<td>Hyperchromasia</td>
</tr>
<tr>
<td>Finely granular chromatin</td>
<td>Mild chromatin irregularity</td>
<td>Coarse chromatin with heterogeneity</td>
</tr>
<tr>
<td>Evenly distributed chromatin</td>
<td>Nucleoli prominent</td>
<td>Occasional nuclei</td>
</tr>
<tr>
<td>Mitosis in repair</td>
<td>Rare mitosis</td>
<td>Occasional mitosis &amp; apoptotic debris</td>
</tr>
<tr>
<td>Abundant cytoplasm</td>
<td>Mild increase N/C ratio</td>
<td>Increased N/C ratio</td>
</tr>
<tr>
<td>Well defined cell borders</td>
<td>Distinct cell borders</td>
<td>Ill-defined cell borders</td>
</tr>
</tbody>
</table>

**Pitfalls of Atypical Endocervical Cells**

- Non-neoplastic processes with atypical cellular changes:
  - Lower uterine segment
  - Tubal metaplasia
  - Repair
  - Endocervical polyps
  - Microglandular hyperplasia
  - Arias-Stella change
  - Radiation changes

**Tubal metaplasia**

- Enlarged nuclei, round to oval
- Pseudostriatification
- Hyperchromasia
- Anisonucleosis ("clumsy" appearance)
- Finely granular, evenly dispersed chromatin
  - Different from AIS: coarse hyperchromasia
- Less common feathering, rosette formation, and mitosis
- Intermixed goblet cells or peg cells
- **Presence of cilia or terminal bars**
- Focal, patchy p16 staining (not diffuse)
- It may coexist with neoplastic lesions
### Mimics of Atypical Glandular Cells

<table>
<thead>
<tr>
<th>CRITERIA</th>
<th>AIS</th>
<th>HSIL</th>
<th>TUBAL MET</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellularity</td>
<td>Cellular</td>
<td>Usually cellular</td>
<td>Rare</td>
</tr>
<tr>
<td>Sheets/stripes</td>
<td>+++</td>
<td>++/+++</td>
<td>+/-</td>
</tr>
<tr>
<td>Crowding/overlap</td>
<td>Present</td>
<td>Present</td>
<td>Present, mild</td>
</tr>
<tr>
<td>Polarity</td>
<td>Present</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Hyperchromasia</td>
<td>Present</td>
<td>Present</td>
<td>Mild</td>
</tr>
<tr>
<td>Nuclear shape</td>
<td>Oval/elongated</td>
<td>Irregular/arrow shape</td>
<td>Oval/cigar shaped</td>
</tr>
<tr>
<td>Feathering</td>
<td>Present</td>
<td>Absent/focal</td>
<td>Rare</td>
</tr>
<tr>
<td>Strips</td>
<td>Present</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Rosettes</td>
<td>Present</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Term.bar/cilia</td>
<td>Absent</td>
<td>Absent</td>
<td>Present/diagnostic</td>
</tr>
<tr>
<td>Mitosis/apoptosis</td>
<td>Present</td>
<td>May be seen</td>
<td>Rare</td>
</tr>
<tr>
<td>p16 pattern</td>
<td>Black positive</td>
<td>Black positive</td>
<td>Patchy positive</td>
</tr>
<tr>
<td>Other</td>
<td>Isolated dysplastic squamous cells</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Modified from Mody DR. Glandular cell abnormalities. Diagnostic Pathology Cytopathology 2014

### HSIL in Glands Pitfall

<table>
<thead>
<tr>
<th></th>
<th>AIS</th>
<th>HSIL</th>
</tr>
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<tbody>
<tr>
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</table>
Atypical Endometrial Cells

- Based on increased nuclear size
- NOT further classified as “favor neoplastic”
  - Difficult and poorly reproducible distinction
- Criteria
  - Small groups
  - Mild nuclear enlargement
  - Mild hyperchromasia
  - Heterogeneous chromatin
  - Small nucleoli may be present (larger in LBP)
  - Scant cytoplasm, vacuolated
  - Ill-defined cell borders
- Associated with EM polyps, chronic endometritis, IUD, EM hyperplasia or EM carcinoma
- Menses: check for LMP, look for “exodus”
- Cell blocks may help in distinction of AGCs (p16)
AGC, Endometrial

AGC Follow-up

- CAP benchmark: reporting rate 0.1-0.2% (50% percentile)
- 25% of AGC test positive for HPV
- Most prevalent HPV genotypes: 18/45, then 16
- 50% of HPV+/AGC associated with significant lesions
  - AIS, ECCa, SIL or SqCC
- <5% of HPV-/AGC associated with significant lesions
  - EMCa or EXUTCa, or EC or EM polyps
- 10-40% of AGC harbor squamous lesions (HSIL/CIN2-3)
  - AIS coexists with HSIL in 50% of cases

Endocervical Adenocarcinoma In Situ

- A non-invasive high grade endocervical glandular lesion
- Nuclear enlargement, hyperchromasia, chromatin abnormality, pseudostratification, and mitotic activity
- Monotonous hyperchromatic nuclei
  - More variability in tubal metaplasia
Endocervical Adenocarcinoma In Situ

- Criteria
  - Numerous dense, cellular hyperchromatic groups at low magnification
  - Sheets, pseudostratified strips and rosettes
  - Nuclear crowding and overlap
  - Loss of honeycomb pattern
  - Palisading nuclear arrangement: Feathering
    - True feathering
    - False feathering
    - “Bird tail-like” arrangement in SurePath
  - Oval, elongated, enlarged nuclei
  - Evenly dispersed, coarse chromatin
  - Small nucleoli
  - Mitosis and apoptotic bodies
  - Increased N/C ratio
  - Diminished cytoplasmic mucin

Loss of honeycomb pattern
Endocervical Cells vs. AIS

AIS: Pseudostratification

ECC
Feathering

• Palisading nuclear arrangement
  – **True feathering**: Nuclei at the periphery protruding beyond the confined of the cell, due to extreme nuclear crowding and cohesion to the basement membrane
  – **False feathering**: Cytoplasmic tufts creating a feathering outline; non-specific and found in benign and premalignant entities
Endocervical Adenocarcinoma

- Cytologic criteria overlaps with AIS
- More pleomorphic nuclei
- Irregular chromatin distribution, clearing
- Nuclear membrane irregularities
- Macronucleoli
- Finely vacuolated cytoplasm
- Necrosis diathesis, clinging diathesis
Endocervical Adenocarcinoma

Endocervical Adenocarcinoma

Endocervical Adenocarcinoma
Endometrial Adenocarcinoma

- Pap tests have low sensitivity for its detection
- Lower cellularity due to exfoliation, not direct sampling
- Nuclei is larger with increasing grade of tumor
- Anisonucleosis
- Lack of polarity
- Moderate hyperchromasia
- Irregular chromatin distribution and clearing (“sieve-like”)
- Nucleoli
- Vacuolated cytoplasm
  - Intracytoplasmic neutrophils (“bag of polyps”)
- Tumor diathesis
Differential Diagnosis

EM carcinoma

ECx carcinoma
EMCa with Clear Cell Features

High grade neuroendocrine carcinoma (SCC)

- Highly aggressive, different management
- Small, uniform, hyperchromatic cells with scant cytoplasm
- Loosely cohesive groups, single cells
- Nuclear molding, crush artefact
- Finely granular chromatin
- Inconspicuous nucleoli
- Strong association with HPV 16/18
- DD: PD SCC, PD Adca, LG ESS, and lymphoma
- Cell block useful: NE markers, p40, p63
EMCa with NE Features

Pap  Synaptophysin

Pitfalls

Small Cell Carcinoma  Follicular Cervicitis
Other Malignant Neoplasms

- Uncommon primary tumors
  - Spindle cell squamous cell carcinoma
  - Poorly differentiated squamous carcinoma with small cells
  - High grade neuroendocrine carcinoma (small cell carcinoma)
    - Strongly associated with HPV 16/18
  - Large cell neuroendocrine carcinoma
  - Low grade neuroendocrine carcinoma (carcinoid tumor)
  - Glassy cell carcinoma
  - Mucinous carcinoma (min deviation adenoca, adenoma malignum)
  - Malignant mixed mullerian tumor
  - Clear cell adenocarcinoma
  - Sarcomas

Other Malignant Neoplasms

- Secondary or metastatic tumors
  - Extrauterine carcinomas:
    - Direct extension: endometrium, bladder and rectum
    - Lymphangiomatous mets: GI, breast, ovary
    - Exfoliated from ovary or malignant ascites
  - Melanoma
    - 5-10% arise in vulva or vagina
    - Primary rare; metastatic more common
  - Lymphoma
    - Diff dx with follicular cervicitis, and small cell carcinoma

Malignant Mixed Mullerian Tumor

- <5% of malignant neoplasms of uterine corpus
- Biphasic: malignant epithelial and mesenchymal components
- Hypercellular
- Pleomorphism
- Coarse granular chromatin
- Macronucleoli
- Necrosis
Mixed Mullerian Tumor

Metastatic Colonic Adenocarcinoma

Metastatic Lobular Carcinoma

TBS Atlas: https://bethesda.soov.wisc.edu/Introduction.htm
Metastatic Ovarian Carcinoma

Melanoma

Melanoma
Adjunctive Testing

- HPV testing
  - Triage management for equivocal specimens
  - Stand alone test for primary screening
- Immunochemical testing
  - Recommendations from recent consensus conference (LAST): biomarkers should be incorporated in histopathology to increase sensitivity and reproducibility of HSIL in biopsies
  - p16 (nucleus and cytoplasmic staining)
  - Ki67 (nuclear staining)
  - ProExC (nuclear staining)
  - Can be used in cell blocks from residual LBP
  - Still not validated by FDA requires lab validation before implementation

FDA-Approved Tests for High-Risk HPV

<table>
<thead>
<tr>
<th>Assay</th>
<th>Qiagen HC2</th>
<th>Hologic Cervista</th>
<th>Roche Cobas</th>
<th>Hologic Aptima</th>
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<tbody>
<tr>
<td>Detection of</td>
<td>HPV DNA</td>
<td>HPV DNA</td>
<td>HPV DNA</td>
<td>HPV E6/E7 RNA</td>
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<tr>
<td># of HPV types</td>
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<td>14</td>
<td>14</td>
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<tr>
<td>Assay type</td>
<td>RNA-DNA hybrids</td>
<td>Invader technology</td>
<td>PCR</td>
<td>RNA amplification/hybridization</td>
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<td>Internal control for specimen adequacy</td>
<td>no</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>HPV 16/18 genotyping available</td>
<td>no</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
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<tr>
<td>Primary Screening</td>
<td>no</td>
<td>no</td>
<td>yes</td>
<td>no</td>
</tr>
</tbody>
</table>

GYN Cytology in the Era of Molecular Testing

Lorincz A. Acta Cytologica 2016;60:501-512
Molecular markers in cervical carcinogenesis

- Methylation
- Mutations
- Intracellular regulatory pathways
- Lineages of cell origin

DNA Methylation as Screening Test

- Strongly associated with CIN and cancer
- Methylation biomarker genes in cervical lesions:
  - CADM1, EPB41L3, FAM19A4, MAL, miR-124, PAX1 & SOX1
- Proposed as a triage for HPV+
  - May complement or replace HPV screening due to its low specificity
- Proposed as indicator of disease progression
  - Elevated quantitative methylation of HPV16L1 & L2 open reading frames associated with CIN2, CIN3 and cancer
  - A biomarker for disease progression will reduce testing, lower costs, fewer overtreatments and less anxiety
- May eliminate cytology in cervical cancer screening

DNA Methylation as Screening Test

- Current triage tests hrHPV+
  - Cytology
  - Immunostaining for p16 and Ki-67
  - Genotyping for HPV16, HPV18, HPV33 & HPV45
- Future triage test
  - DNA methylation testing
- Methodologies
  - Quantitative methylation-specific PCR (QMSP)
  - Pyrosequencing
  - Parallel deep-sequencing
DNA Methylation as Screening Test

- Three basic target sequence designs
  - HPV genomes
  - Human genomes
  - Combination HPV and human DNA
- Not enough information on which classifier will yield the best sensitivity for CIN3
- Theory: Absence of detectable methylation in CIN2-3 would identify high grade lesions that would not progress