ThinPrep Pap Test Morphology Overview

- History of the ThinPrep Pap Test
- Specimen Adequacy
- Normal Morphology
- Abnormal Morphology
History of the ThinPrep Pap Test

Where it all began

• Cytyc’s initial development efforts focused on Imaging (1988)
• ThinPrep Pap Test FDA approved 1996
• Sample/Preparation Limitations Addressed
ThinPrep® Process

Dispersion  Collection  Transfer
Pap Test Methods

Conventional Smear

SurePath

ThinPrep Pap
Pap Test Methods

ThinPrep vs Conventional

Similarities

• Classic Cell Morphology
• Assessment of Cellularity
• Slow, Systematic Screening

Differences

• Collection
• Immediate Fixation
• Thin Layer

Pap Test Methods

ThinPrep vs SurePath

**Similarities**

- Liquid based
- Thin layer

**Differences**

- Collection
- Fixative
- Sample Transfer
ThinPrep Screening Tips

- Slow
- Systematic
- Overlap
- Background Awareness
# The Bethesda System for Reporting Cervical Cytology

## Adequacy

<table>
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<tr>
<th>FN20 Eyepiece/10x Objective</th>
<th>FN20 Eyepiece/40x Objective</th>
<th>FN22 Eyepiece/10x Objective</th>
<th>FN22 Eyepiece/40x Objective</th>
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<td>50.0</td>
<td>3.1</td>
<td>60.5</td>
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5,000 Well Preserved, Visualized Squamous Cells

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Adequacy- Suggested Counting Method #1

Adequacy- Suggested Counting Method #2

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Adequacy- Other Considerations

- Atrophy
- Chemo- or radiation therapy
- Post-hysterectomy

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Adequacy - Obscuring Factors and Interfering Substances

- Lubricant
- Blood
- Mucus
- Inflammation

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Adequacy- Obscuring Factors and Interfering Substances

Unsatisfactory

- Greater than 75% squamous cells obscured AND no abnormal cells

Satisfactory

- 50-75% squamous cells obscured
  - Include statement describing the specimen as partially obscured

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Adequacy - Obscuring Factors and Interfering Substances: Examples of Lubricant
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Adequacy- Obscuring Factors and Interfering Substances: Examples of Blood, Mucus, Inflammation

Blood

Mucus

Inflammation
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Endocervical/Transformation Zone Component

- Not necessary for an adequate specimen
- 10 well-preserved endocervical or squamous metaplastic cells
- Singly or in clusters

ThinPrep Pap Test:
Normal Morphology
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Normal Morphology – Squamous Metaplasia

• Singly or in groups (Sheets/cobblestone arrangement)
• Nuclei 1-2x size of intermediate cell nucleus ~50 microns
• N/C ratio may be variable
• Smooth nuclear membranes
• Finely granular and evenly distributed chromatin
• Dense, homogenous cytoplasm may be vacuolated
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Normal Morphology – Endocervical Cells

• Singly or in groups
• “Honeycomb” or “picket-fence” arrangements
• Nuclear size is highly variable
• Smooth nuclear membranes
• Finely granular and evenly distributed chromatin
• Cytoplasm is diffusely vacuolated or granular
• Small nucleoli may be present
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Normal Morphology – Tubal Metaplasia

• Pseudostratified crowded groups with maintained polarity
• Terminal bars and cilia
• Nuclei are round to oval and may be enlarged, pleomorphic, and often hyperchromatic
• N/C ratio can be high
• Cytoplasm may show discrete vacuoles or goblet cell change
• Nucleoli are not usually seen
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Normal Morphology – Endometrial Cells

- Dense 3-dimensional groups, exodus ball, or loose aggregates
- Nuclei with dense, heterogenous chromatin
- Nuclear size slightly smaller than benign intermediate cell nucleus (~ 35 µm²)
- Nuclear membranes may be irregular
- Cytoplasm is scant, dense, or vacuolated

Endometrial Cells

Cuboidal Endocervical Cells
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Normal Morphology – Lower Uterine Segment (LUS)

- Columnar in shape and may present in tubular formation
- Nuclear crowding and overlapping with polarity maintained
- Nuclei are small, round to oval, and variably hyperchromatic
- Smooth nuclear membrane
- Moderately coarse, evenly distributed chromatin
- Scant, spindled cytoplasm
- Inconspicuous nucleoli may be present
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Normal Morphology – Lymphocytic (Follicular) Cervicitis

- Polymorphous population of lymphocytes
- Loose aggregates or scattered single cells in the background
- Tingible body macrophages may be present

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Normal Morphology – Parabasal Cells (Atrophy)

- Single cells and/or flat monolayer sheets with preserved nuclear polarity
- Oval nuclei with finely granular evenly distributed chromatin
- Smooth nuclear membranes
- Nuclear size approximately 50 µm²
- N/C ratio increased compared to intermediate/superficial cells
- Cytoplasm is more granular and dense
- Naked nuclei may be present
Normal Morphology – Trichomonas

- Pear-shaped, oval and/or round organisms from 15 to 30 µm²
- Eccentrically located pale, vesicular nuclei
- Eosinophilic cytoplasmic granules
- Squamous cells with perinuclear halos
Trichomonas

Cytoplasmic Blob
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Normal Morphology – Candida

• Pseudohyphae of various lengths
• Budding yeast (3-7 µm²)
• “Spearing” of epithelial cells, often seen on low power
• Reactive squamous cells with mild nuclear enlargement may be seen
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Normal Morphology – Actinomyces

- Thin and thick filaments with radial distribution
- Tangled clumps of filamentous organisms
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Normal Morphology – Herpes

- Nuclei with “ground-glass” appearance
- Multinucleation
- Margination of chromatin
- Nuclear molding
- Eosinophilic nuclear inclusions

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Normal Morphology – Shift in Flora (Bacterial Vaginosis - BV)

• Squamous cells covered with coccobacilli - “clue cells”
• Cloudy, filmy appearance
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Normal Morphology – Reactive/Repair

- Cells present in cohesive sheets with maintained polarity ("school of fish")
- Nuclear size variable
- Nuclear membranes are smooth, round, and uniform
- Chromatin is finely granular and evenly distributed
- Prominent single or multiple nucleoli may be seen
- Cytoplasmic boundaries are well defined
Repair

Non-Keratinizing Squamous Cell Carcinoma
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Normal Morphology – Radiation Changes

• Cell size markedly increased without substantial increase in N/C Ratio
• Bizarre cell shapes
• Binucleation and multinucleation
• Enlarged nuclei with degenerative/smudgy chromatin or mild hyperchromasia
• Prominent single or multiple nucleoli may be seen
• Cytoplasmic vacuoles and/or polychromasia may be seen

PATIENT HISTORY IS KEY
ThinPrep Pap Test:

Abnormal Morphology
Abnormal Morphology – Atypical Squamous Cells of Undetermined Significance (ASCUS)

- Singly or sheets
- Nuclei 2 ½ to 3 times the area of an intermediate nucleus
- Slight increase in N/C ratio
- Mild irregular nuclear membranes
- Minimal hyperchromasia
- Mild irregular chromatin distribution
- Cytoplasm can be keratinized and/or exhibit poorly defined cytoplasmic halos or vacuoles
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Abnormal Morphology – Low Grade Squamous Intraepithelial Lesion (LSIL)

• Cells occur singly, in clusters, and in sheets
• Low but slightly increased N/C ratio
• Nuclei more than 3 times the area of an intermediate nucleus
• May be hyperchromatic
• Chromatin is uniform and coarsely granular, smudgy and/or opaque
• Variable nuclear membranes
• Koilocytosis/perinuclear cavitation
• Binucleation and/or multinucleation
• Nucleoli absent or inconspicuous if present

LSIL

Navicular Cells
Abnormal Morphology – Atypical Squamous Cells- Cannot Exclude HSIL (ASC-H)

• Occurs singly or in small groups of less than 10 cells
• Nuclei 1½ to 2½ times the area of a normal metaplastic nucleus
• N/C ratio similar to that of HSIL
• Chromatin irregularity
• Hyperchromasia
• Irregular nuclear membranes

CRITERIA INSUFFICIENT FOR HSIL DIAGNOSIS
ASC-H

Reactive Squamous Metaplasia
Abnormal Morphology – High Grade Squamous Intraepithelial Lesion (HSIL)

• Appear singly, in sheets, or in syncytial-like aggregates
• High N/C ratio
• Variable nuclear size
• Chromatin can be fine or coarse and evenly distributed
• May appear hyper- or hypochromatic
• Irregular nuclear membrane with frequent indentations/grooves
• Cytoplasm can appear “immature”, lacy, and delicate, densely metaplastic, or keratinized
• Nucleoli generally absent but may occasionally be seen
HSIL Parabasal Cells
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Abnormal Morphology – Squamous Cell Carcinoma

- Isolated single cells or syncytial aggregates with poorly defined cell borders
- Nuclei vary markedly in size and shape and may be densely opaque
- Irregular nuclear membranes
- Chromatin may appear coarsely granular, irregularly distributed with chromatin clearing
- Nucleoli may be prominent, but less common in Keratinizing Squamous Cell Carcinoma
- Caudate/spindle cells
- May be keratinized
- Tumor diathesis
Non-Keratinizing Squamous Cell Carcinoma

Endocervical Adenocarcinoma
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Abnormal Morphology – Atypical Endocervical Cells, NOS

- Sheets, strips and/or 3-dimensional clusters with cell crowding, nuclear overlap and/or pseudostratification
- Slight variation in nuclear size and shape
- Nuclear enlargement, up to 3 to 5 times normal
- Mild nuclear hyperchromasia and chromatin irregularity
- Occasional nucleoli
- Cytoplasm may be abundant with distinct cell borders

AGC Endocervical, NOS

HSIL with Glandular Involvement
AGC Endocervical, NOS

Tubal Metaplasia
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Abnormal Morphology – Atypical Endocervical Cells, Favor Neoplastic

- Appear in sheets and strips with nuclear crowding, overlap, and/or pseudostratification
- Rare cell groups with rosettes or feathering
- Nuclei are enlarged and often elongated with some hyperchromasia
- Variable coarse chromatin
- Increased N/C ratio
- Poorly defined cell borders

CRITERIA INSUFFICIENT FOR AIS OR INVASIVE ADENOCARCIMONA

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Abnormal Morphology – Atypical Endometrial Cells

- Small 3-dimensional clusters (usually 5-10 cells per group)
- Nuclei are slightly enlarged compared to normal
- Chromatin variability
- Mild hyperchromasia
- Occasional small nucleoli
- Scant, vacuolated cytoplasm

ATYPICAL ENDOMETRIAL CELLS ARE NOT FURTHER QUALIFIED

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Abnormal Morphology – Endocervical Adenocarcinoma In Situ (AIS)

- Hyperchromatic crowded groups (HCG)
- Pseudostratified strips and/or rosettes
- Palisading nuclear arrangement- “feathering”
- Nuclear enlargement with crowding and overlap
- Oval or elongated nuclei of variable sizes
- Hyperchromatic nuclei with evenly dispersed, coarsely granular chromatin
- Small or inconspicuous nucleoli
- Mitotic figures common
- Absence of tumor diathesis

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Abnormal Morphology – Endocervical Adenocarcinoma

- Single cells, sheets, and/or 3-dimensional clusters with cell crowding
- Nuclear enlargement with pleomorphism
- Fine or coarsely granular but irregularly distributed chromatin
- Irregular nuclear membranes
- Prominent nucleoli
- Vacuolated cytoplasm
- Tumor diathesis
Endocervical Adenocarcinoma

Non-Keratinizing Squamous Cell Carcinoma
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Abnormal Morphology – Endometrial Adenocarcinoma

• Single cells and/or small 3-dimensional clusters with cell crowding
• Variable nuclear enlargement
• Fine or coarsely granular, slightly irregular chromatin
• Small to prominent nucleoli
• Vacuolated cytoplasm
• Poly engulfment may be present
• Tumor diathesis – may be finely granular or “watery”
Thank you for your participation

Contact Information
[Email address – enter presenter information]
[Phone number – enter presenter information]

Additional Resources
www.hologic.com
www.cytologystuff.com