ThinPrep® Morphology
ThinPrep® Morphology

Normal Cytology
CT & Pathologist Training

*Training program begins with ThinPrep® morphology presentation*

- Microscopic training sessions for individual and multi-headed group screening sessions are designed to develop and refine screening and interpretive skills.
- Screening Evaluation modules provide more thought-provoking cases intended to provide a full range of diagnostic challenges.
- Competency Assessment modules are used to monitor training participants’ performance (pre- and post-tests).
- Laboratory training is the final phase of training within the laboratory. The goal for the Pathologist/CT is to transfer conventional pap knowledge and confidence by screening and diagnosing ThinPrep slides. Ongoing laboratory training material (glass slides) comes from ThinPrep Pap Test collection in the laboratory.
ThinPrep® Process

*Three key phases*

1. **Dispersion:** Randomizes/homogenizes patient’s cell population within the vial.

2. **Cell Collection:** ThinPrep software monitors the flow rate and senses when pores in the filter are blocked by material (red blood cells [RBCs], white blood cells [WBCs], and epithelial cells).

3. **Cell Transfer:** The cylinder is inverted and the filter comes in contact with the glass slide. Air pressure from behind the filter aids the cells’ natural attraction to the glass slide.
Conventional Pap Smear (Macroscopic)

- The limitations of the manual smearing method are apparent, with variable thickness of the smear. Fortunately, conventional slide morphology is transferable to the ThinPrep® process. Cytology knowledge does not have to be relearned, base knowledge is merely refined.
ThinPrep® Pap Test (Macroscopic)

- A key difference in presentation is the absence of a manual smear pattern. The ThinPrep process takes the patient sample and applies it onto the center of the slide in a thin, uniform layer. Specimen preparation is standardized, eliminating the inconsistency associated with manual preparations.
Conventional Pap (CP) smear

- Microscopically, the uneven distribution of cellular material associated with the CP smear pattern is evident.
ThinPrep® (TP) Pap Test

Same Patient

- Tissue architecture is maintained but the ThinPrep process rearranges the relationship of cell groups and provides a more even distribution of cells and groups on the glass slide. Note the group/sheet of intact endocervical cells.
Morphology I  

Slide: 9

ThinPrep® Characteristics

- Liquid Based Fixation
- Cell Size
- Smear Pattern
- Specimen Background
- Similarities > Differences

TP Characteristics

Common changes associated with TP morphology

- Liquid Based Fixation - The key difference.
- Cell Size - Related to fixation and the effect of cells placed into solution.
- Smear Pattern - No longer smearing cellular material across the glass slide.
- Specimen Background - Unique characteristics of rinsing cells into a solution but “clues” are still present.

Most important, the similarities between the TP and CP far outweigh the differences.
ThinPrep® Characteristics

Liquid Based Fixation

• Enhanced Cytoplasmic Detail

• Enhanced Nuclear Detail

• Variability in Nuclear Staining

TP Characteristics

Liquid Based Fixation

• Enhanced cytoplasmic detail – Optimized fixation, lack of mechanical (smearing) artifact.

• Enhanced nuclear detail - Optimized fixation, lack of mechanical (smearing) artifact.

• Variability in nuclear staining – The chromatin detail is more readily appreciated and can appear as hypo- and hyperchromatic nuclei within the same case.
TP Characteristics

Cytoplasmic Detail

- Koilocytosis caused by HPV is often more evident than in conventional paps and the spectrum of eosinophilic and cyanophilic staining is seen. Mechanical artifacts are absent.
TP Characteristics

Nuclear Detail

- Dark, hyperchromatic nuclei are still identified on TP, as illustrated in this HSIL cell.
TP Characteristics

Variability in Nuclear Staining - ThinPrep® Stain

- Left image: Dependent upon patient biology, the chromatin of the dysplastic cells can appear hypochromatic. However, increased N/C ratios and irregular nuclear membranes should alert you to an abnormal process, as in this case of HSIL.
- Right image: These HSIL nuclei show the hyperchromatic chromatin pattern typically associated with dysplasia.
TP Characteristics

*Variability in Nuclear Staining – Richard-Allan stain*

- Left image: Here is another example of hypochromatic appearing nuclei in a case of HSIL stained with Richard-Allan stain. It is important that these cells are not missed, regardless of the stain used.
- Right image: These HSIL nuclei show hyperchromatic chromatin.
ThinPrep® Characteristics

Cell Size

- “Proportionately” Smaller
- Single Cells More Prominent
- Cells Round-Up in Solution

TP Characteristics

Cell Size

- “Proportionally” smaller cells - Due to the inherent difference with alcohol fixation and the elimination of air-drying artifact cells may appear smaller but will be in proportion to the other cells on the slide.
- Single cells are more prominent - Single cell populations are not created, just more noticeable in the background.
- Cells round up in solution – Due to the physical properties related to suspension in fluid, the changes are similar to a non-gyn presentation.
TP Characteristics

Single Cells

- Single cells and small groups are easily seen because of the thin layer presentation. These include endocervicals, endometrials and squamous metaplastic cells.
TP Characteristics

Proportionately Smaller/Rounded Up

- The squamous metaplastic cells in this cluster may appear to be smaller than those on conventional paps but are proportional in size to the mature squamous cells in the background. There is also more depth of focus to the group due to the rounding up which occurs in solution.
**ThinPrep® Characteristics**

**Smear Pattern**
- Mechanical Artifact Eliminated
- Cellular Material Not Pulled Out in Mucus
- Tissue Architecture Maintained

**TP Characteristics**

*Smear Pattern*

- Mechanical artifacts associated with pulling the cells across the slide have been eliminated.
- Cellular material is not pulled out in mucus, therefore there is a new pattern recognition that needs to be established for TPs. Endocervical cells in particular will be distributed throughout the cell deposit and are not caught up in mucus.
- Tissue architecture is maintained throughout the slide processing. Architecture is not disrupted, only the relationships of the cell groupings have been altered.
**TP Characteristics**

*CP – Smear Pattern*

- This slide exhibits a common presentation of cells pulled or streaked across the slide with areas of obscuring inflammation and mucus.
**TP Characteristics**

*TP – Same Patient*

- Notice that the cells are evenly distributed throughout the background, not pulled out in mucus.
- Mechanical artifact is eliminated.
- White cells, indicating that inflammation is still present, can be seen in the background.
**ThinPrep® Characteristics**

**Specimen Background**
- Cleaner Background
- Cellular Debris More Clumped
- “Clues” in the Background
- “RATTY” Background:
  Infectious agent, Cytolysis, Blood, Disease

**TP Characteristics**

*Specimen Background*

- Specimen background appears cleaner as a result of the ThinPrep® process.
- Fluid collection may cause cellular debris to clump.
- Background can provide clues. TP requires more vigilance when screening because the cellular and non-cellular elements typically obscured on the CP are more easily identified on a TP slide and there is more to see per field.
- “Ratty” background can be advantageous during screening and infectious agents, cytolysis and/or disease should be considered. Tumor diathesis, blood and cytolysis will still be present on the slide and should be used in the differential diagnosis.
Specimen Background

*Cytolysis*

• Bare nuclei and cytoplasmic debris are evenly dispersed across the cellular deposit. Döderlein bacilli typically seen on top of squamous cells or trapped in mucus are still present.
Specimen Background

*Blood*

- RBCs appear as ghost-like cells as a result of the blood lysing properties of the preservative solution. Hemolyzed blood is often found clumped.
Specimen Background

*Trichomonas vaginalis*

- Well preserved organisms along with a bacterial background can give the appearance of a “ratty” specimen.
Specimen Background

Tumor Diathesis

- Tumor diathesis retains the characteristic appearance of cell debris, protein and blood in the background in addition to WBC’s. It also maintains the dirty/gritty background appearance seen on a CP.
Microscopic Evaluation

When Screening Slides:

- Systematic approach - Screening vigilance is required over a smaller area with an increased attention to the background where potential single abnormal cells may be found.
- Slow approach – A natural tendency may be to screen fast because of the cleaner appearance of a TP but a slow, steady screening technique allows time to incorporate subtle background clues. Cleaner background does not necessarily indicate normal cell population.
- Slight overlap – This technique is still required to ensure that as many cells as possible can be visualized during screening.
Specimen Adequacy

Cellular Composition

Specimen adequacy guidelines for Liquid Based Cytology as recommended by the Bethesda System 2001 are:

- 5000 well-visualized and preserved squamous cells are necessary for a satisfactory specimen.
- Strict objective criteria may not apply in every case. Slides with cell clustering, atrophy or cytolysis are technically difficult to count and laboratories should apply professional judgment and employ hierarchical review when evaluating these slides.
- The presence or absence of an endocervical component should be noted. Ten well-preserved endocervical or squamous metaplastic cells, singly or in groups, should be observed to report a transformation zone component as present.
- The presence of abnormal cells makes any specimen an adequate specimen.
Depicted is a 10X field containing 60 squamous cells.

- Satisfactory field @ FN22
- TBS requirements are:
  - 10 fields across cell deposit diameter should have an average of 60 cells each.
Depicted is a 10X field containing 50 squamous cells.

- Satisfactory field @ FN20
- TBS requirements are:
  - 10 fields across the cell deposit diameter should have an average of 50 cells each.
Depicted is a 10X field containing 40 squamous cells.

- Unsatisfactory field due to insufficient squamous epithelial component
Cellular Composition

*Unsat/Blood*

- Predominantly blood was rinsed into this PreservCyt® solution vial. Microscopically only lysed blood, rare epithelial cells, and ghost RBCs are identified and this slide would be unsatisfactory for interpretation. Communication between the lab and the clinician remains key in troubleshooting unsat specimens.
Cellular Composition

Unsat/WBCs

- RBCs, WBCs and epithelial cells all compete for a place on the filter, so if there is a heavy inflammatory population, that will alter the composition of the final TP slide. The slide is still representative of what the clinician has collected and rinsed into the vial.
Cellular Composition

*Unsat/Mucoid*

- Mucus will compete with cells for a spot on the filter. A truly mucoid specimen will show islands or pools of mucus and may affect the specimen adequacy.
Endocervical Cells

- Honeycomb/palisading arrangements maintained.
- Round-up in solution.
- More tightly packed groups.
- Smaller cell groups/single cells.
- Nuclei “busier”.

Endocervical Cells

- Honeycomb/palisading formations are maintained.
- Cells tend to round up in solution.
- More tightly packed cell groupings with cell groups twisted or folded from the fluid collection method may be seen.
- Smaller endocervical cell groupings as well as single cells may be present and careful screening is required to identify them.
- Liquid based fixation results in nuclei that appear busier, more reactive.
TP Characteristics

Endocervicals

- Large sheet of endocervical cells exhibiting both honeycomb and picket-fence arrangements.
- Nuclei are small and uniform.
TP Characteristics

*Endocervicals*

- These cells exhibit traditional palisading formation with small, basally located nuclei.
TP Characteristics

*Endocervicals*

- Small, cuboidal group of endocervical cells with round to oval uniform nuclei can be seen. Infrequent, small cell groupings may be more challenging to find and identify.
Squamous Metaplasia

- Traditional sheets and cobblestone arrangements are presented.
- Typical dense, homogenous cytoplasm is evident. Increased cytoplasmic vacuolization may be present as a result of the liquid based fixation.
- Metaplastic cells can often occur singly.
- Cells appear smaller and rounder as a result of the fluid collection.
TP Characteristics

*Squamous Metaplasia*

- Small grouping of squamous metaplastic cells with dense cytoplasm, cytoplasmic vacuolization, and uniform, round nuclei.
- “Pseudopodia” or cytoplasmic processes identified on the conventional smear are still evident even with the tendency of cells to round up in solution.
TP Characteristics

Squamous Metaplasia

- As a result of the liquid fixation, squamous metaplasia may present as larger sheets with more depth to the groupings. There are prominent cytoplasmic borders with some cytoplasmic vacuolization and round to oval nuclei with smooth nuclear membranes, fine, even chromatin and micronucleoli.
Endometrial Cells

- Tight 3D Cell Clusters
- Loose Cell Clusters with Vacuolated Cytoplasm
- Single Cells More Prominent
- Nuclei “Busier”
- Menstrual Blood Lysed

Endometrial Cells

- Tight 3D cell clusters are maintained.
- More loose cell groupings with cytoplasmic vacuolization may be identified.
- Single cells are more prominent against a cleaner background.
- Nuclei appear busier as a result of the improved preservation in the liquid based fixative.
- Menstrual blood in the background is lysed, and may appear clumped.
TP Characteristics

*Endometrials*

- Menstrual background - RBCs appear hidden in background as ghost blood cells as a result of the lysing properties of the preservative solution. This lysed blood may have a tendency to present in clumps.
TP Characteristics

*Endometrial Cells*

- Tight cell group with endometrial and stromal cells typical of an exodus pattern.
TP Characteristics

Endometrial Stromal Component

- A small loose cluster with kidney bean shaped nuclei and vacuolated cytoplasm. Note the size relationship of the stromal cells to the intermediate cell nuclei.
Look-Alike

*Endocervical vs. Endometrial*

- Left image: The grouping of small endocervical cells presents in a flat sheet with nuclei of equal size and shape.
- Right image: The endometrial cells maintain a 3D configuration and the nuclei may vary in shape but do not vary in size.
Atrophy

Preservation is greatly enhanced as a result of the liquid based fixation with the elimination of the air-drying artifact commonly seen on CP.

- Consistently observe sheets of well preserved parabasal cells.
- It is possible to distinguish endocervical cells from parabasal cells, often something that was difficult on a conventional slide.
- The number of bare nuclei are reduced by removing the mechanical artifact of smearing.
- “Atrophic vaginitis” background pattern appears more clumped as a result of the fluid collection method.
Parabasal Cells

- Parabasal cells may appear in uniform large sheets, with easily identifiable cytoplasmic borders, abundant cytoplasm and round to oval nuclei. Sheets may be twisted or folded over during the preparation process.
Atrophy

- Sheets and single parabasal cells are readily identified.
- Stripped parabasal nuclei are reduced but not eliminated.
Atrophy

- Squamous component may be easily distinguished from the honeycombed endocervical component.
Atrophic Vaginitis

- The background pattern presents as clumped cellular debris and degenerated WBCs encircling the parabasal cells.
Trichomonas vaginalis

- Frequently Smaller
- Internal Structure Readily Identifiable
- Classic “Trich” Pattern Maintained

Trichomonas vaginalis

- Organisms may appear smaller as a result of the liquid based fixation.
- It is easier to identify the internal structure to differentiate cytoplasmic debris from an organism.
- The classic “Trich” pattern is maintained and inflammatory cell changes are still identifiable.
Trichomonas vaginalis

- Inflammatory cell changes, including peri-nuclear halos, cytoplasmic vacuolization and bichromatic staining are useful in pattern recognition. The well preserved organism can readily be distinguished from cytoplasmic debris (look-alike) by visualizing the elongated eye-spot within the pear-shaped organism. Flagella may also be preserved.

(Compare the trichomonad to the adjacent white cell).
*Trichomonas vaginalis*

*(Screening Power)*

- Even in the absence of inflammatory cellular changes, the organisms (about the size of the WBCs) can be noted in the background.
Candida spp.

- Classic Cell Clumping
- Reactive Squamous Cells with Engulfed WBC’s
- Distinguish Mucus Strands from Pseudohyphae

Candida Spp.

- Classic cell clumping is more evident.
- Reactive squamous cells with engulfed WBCs may be present as a clue.
- It is important to differentiate between mucus strands and pseudohyphae.
**Candida Spp.**

*(Low Power)*

- Organism can be seen isolated in the clean background or in the traditional “shish-kabob” or “spearing” arrangement.
Reactive Changes Due to Inflammation

- Reactive squamous cells with engulfed WBCs associated with *Candida* spp. often serve as a clue and present with vacuolated “tissue paper” or “moth-eaten” cytoplasm and reactive nuclei.
Look-Alike

*Candida vs. Mucus*

- Left image: *Candida*, like mucus strands, can be twisted within the cell groupings. However, the pseudohyphae can be identified projecting from the groupings and distinct cell walls can be seen.

- Right image: Mucus strands can be seen extending from cell groups but, upon close inspection, the strands are irregular in width and distinct hyphae and spores are not present.
Candida Spp.

- Fungal spores are retained and tend to clump together or pile on top of cells.
Actinomyces

- Organized Clusters of Branching Filamentous Bacteria
- Associated Blue Staining Bacteria

*Actinomyces*

- Organized clusters of branching filamentous bacteria.
- Associated blue staining bacteria are present.
**Actinomyces**

- Classic blue staining bacteria associated with the branching filamentous bacteria can be seen.
- Most often associated with patients with an IUD.
Herpes Simplex Virus

- Ground Glass Nuclei
- Multinucleation with Nuclear Molding
- Classic Eosinophilic Nuclear Inclusions

**Herpes Simplex Virus**

*Classic morphology, cells groupings are clearly noted from screening power, isolated by the relatively clean background.*

- Diagnostic ground glass nuclei are present with chromatin margination and sharply defined nuclear membranes.
- Multinucleation with nuclear molding is easily appreciated.
- Eosinophilic nuclear inclusions, when present, are evident.
Herpes Simplex Virus

- Multinucleation with nuclear molding and ground glass nuclei are seen in these cells. Nuclear inclusions are more prominent with liquid based fixation. Drying and/or mechanical artifacts are eliminated.
**Inflammation**

*It is important to use all criteria for a diagnosis.*

- Slight nuclear enlargement (2x intermediate cell nuclei).
- Perinuclear halos – Important to distinguish from koilocytosis associated with HPV changes.
- Cytoplasmic vacuolization and bi-chromasia may be present, similar to morphology as seen on CP.
- Clumped WBCs in background are more visible.
- Enhanced cellular detail is present due to the improved fixation and preparation.
Inflammatory Changes

- These cells exhibit a slight increase in nuclear size and hyperchromasia, with some nucleoli present. Perinuclear halos and bi-chromasia are also noted. Nuclear changes fall short of those required for an ASC-US diagnosis.
- Slight nuclear membrane irregularities are somewhat uniform in appearance and are a direct result of cytoplasmic degenerative changes.
Reactive Changes:

**Repair**

- Sheets of Cells with Engulfed WBC’s
- Sheets of Cells More Rounded
- Greater Depth-of-Focus
- Sheets of Cells Less “Pulled Out”

**Repair**

- Sheets of epithelial cells are present with engulfed WBCs.
- Cell groupings are more rounded and display a greater depth-of-focus due to the liquid based fixation.
- Mechanical artifact is eliminated and the cells don’t appear as “pulled out.”
Repair

- Nuclei in this group of repair show bland chromatin, regular nuclear membranes, nucleoli, uniformity within the sheet, and maintain nuclear polarity.
**Look-Alike: Repair vs. Carcinoma**

- Repair maintains a sheet-like configuration with a tissue-culture appearance to the cytoplasm. Nuclei may be enlarged, however, chromatin pattern is evenly distributed and nuclear borders are smooth. Nuclei maintain a polarity to each other in these groups. Nucleoli are present, may be prominent but are not in every nucleus.
- Carcinoma shows a greater depth of focus to the cell cluster and greater variability from nucleus to nucleus with a marked alteration in polarity. Nucleoli will be prominent and often multiple.
ThinPrep® Morphology

Epithelial Cell Abnormalities
**ThinPrep® Characteristics**

- Liquid Based Fixation
- Cell Size
- Smear Pattern
- Specimen Background
- Similarities > Differences

**TP Characteristics**

*Common changes associated with TP morphology*

- Liquid Based Fixation - The key difference.
- Cell Size - Related to fixation and the effect of cells placed into solution.
- Smear Pattern - No longer smearing cellular material across the glass slide.
- Specimen Background - Unique characteristics of rinsing cells into a solution but “clues” are still present.

Most important, the similarities between the TP and CP far outweigh the differences.
Epithelial Cell Abnormalities - Squamous

ASC-US

- Occur in Sheets or Singly
- Nuclei 2 ½ - 3X Intermediate Nucleus
- Uniform Chromatin Distribution
- Decrease in Preparation Artifact

**ASC-US**

*ThinPrep reduces preparation artifact associated with some ASC-US*

- Cells are found in sheets or singly
- Nuclei $2^{1/2} – 3$ times the size of an intermediate nucleus
- Uniform chromatin distribution
- Decrease in preparation artifact due to liquid based fixation

As a general guide, the frequency of ASC-US diagnoses should not exceed 2 – 3 times the rate of SIL.
ASC-US

• The increase in nuclear size, increased hyperchromasia and chromatin clumping shown here qualitatively and quantitatively falls short of true LSIL.
ASC-US

- Small group of squamous cells exhibiting slight nuclear enlargement and a suggestion of HPV.
ASC-US

- A group of squamous cells representing atypical parakeratosis with slightly enlarged, irregular, hyperchromatic nuclei.
ASC-H

- Immature metaplastic cells with increased N/C ratios, slight nuclear membrane irregularities and nuclear size variability, suggestive of but not definitive for HSIL.
LSIL

With ThinPrep, the decision between normal and abnormal is more straightforward. The ASC-US category still exists but the liquid based fixation allows for better visibility of cellular criteria and may result in a definitive LSIL diagnosis rather than an equivocal ASCUS diagnosis.

- Increased nuclear detail results in more chromatin detail visible within nuclei.
- Nuclear membrane irregularities are more evident, otherwise lost or obscured on conventional slide due to thick smear, air-drying artifact or obscuring inflammation.
- Sharp, irregular cytoplasmic cavitation (HPV effect).
LSIL

• This sheet of squamous cells exhibits well defined LSIL criteria: nuclear enlargement, hyperchromasia, binucleation and cytoplasmic cavitations
LSIL

• These mature squamous cells show dark, hyperchromatic nuclei in comparison to the normal cell population. Upon closer inspection, the nuclear membranes exhibit irregularities.
LSIL

*HPV Effect*

- Sharp, irregular cavitations in the cytoplasm are the most prominent feature of these cells. Increased chromatin clumping is evident as is a smudged nuclear appearance, secondary to HPV.
LSIL

• On screening power it’s easy to see abnormal groups with hyperchromasia, nuclear enlargement and HPV changes.
Look-Alike: HPV Cavitation vs. Vacuoles

- Left image: Dense, sharp and irregular cavitations caused by viral particles are evident. Cells infected with HPV have a “fried egg” (flat) appearance as opposed to:
- Right image: which shows the smooth bordered glycogen vacuoles. If present, the glycogen may have a waxy appearance. These cells have a greater depth of focus with a bloated appearance.
Epithelial Cell Abnormalities - Squamous

**HSIL**

- Sheets & Syncytial Groupings Maintained
- Cytoplasmic Borders More Distinct
- Isolated, Immature Cell Forms; Function as “Clue”
- Nuclear Membrane Irregularities

**HSIL**

- Sheets and syncytial groupings are maintained, not disrupted by the dispersion process
- More distinct cytoplasmic borders are present and more information can be gained from the cytoplasmic appearance
- Isolated immature cell forms present in background function as “clue”
- Nuclear membrane irregularities are more prominent
HSIL

- Metaplastic type squamous cells showing increased N/C ratios (in excess of 50%), nuclear size and shape variability, nuclear membrane irregularities and coarse chromatin.
HSIL

- Dense immature cytoplasm, increased N/C ratios, and nuclear size and shape variability result in a diagnosis of HSIL.
HSIL

- At low power, abnormal single cells will serve as a “clue” and upon further inspection, additional cells, both singly and in groups, will aid in making the diagnosis.
HSIL

• Upon closer inspection this small sheet of immature squamous cells exhibit scant cytoplasm and hyperchromatic nuclei with coarse but evenly distributed chromatin.

• These immature squamous cells are small and, during screening, attention to the background cell population is necessary.
Look-Alike: HSIL vs. Endometrials

• Left image: Groups of HSIL cells have less depth-of-focus, more distinct cytoplasmic borders and greater nuclear variability.

• Right image: Endometrial cells present as 3 dimensional groups with small, tightly packed nuclei that vary in shape but not size. The nuclear features are consistent within the group and may frequently be accompanied by menstrual blood.

*Close inspection (on high power) is crucial to definitively identify HSIL from benign endometrials.*
Look-Alike: HSIL vs. Atrophy

• Left image: Immature squamous metaplastic cells are present exhibiting increased N/C ratios, hyperchromatic nuclei with nuclear membrane irregularities and coarse chromatin.

• Right image: These parabasal cells show uniformity from nucleus to nucleus as well as a bland chromatin pattern to aid in the determination of a benign origin.
Squamous Cell Carcinoma

- Tumor diathesis is maintained with the distinctive pattern of proteinaceous debris, engulfed white blood cells, degenerated cellular material and, in a case of keratinizing squamous cell carcinoma, increased keratinized debris.
- Greater depth-of-focus to cell groups.
- Nuclei present with variability in chromatin pattern often with parachromatin clearing.
- Nucleoli, if present, may be prominent.
Squamous Cell Carcinoma

- Diathesis is present as necrotic debris in the background mixed with protein, blood, and white blood cells. It is important to identify the abnormal cellular component in the debris. Use the dirty/ratty background as a contextual clue to alert you to the presence of disease.
Squamous Cell Carcinoma

- Dense, angular cytoplasm helps to identify this group of malignant cells as squamous in differentiation. Prominent nucleoli are present, with irregular chromatin distribution and thickened nuclear membranes.
Squamous Cell Carcinoma

- Non-keratinizing squamous cell carcinoma can present with pale immature cytoplasm. These nuclei have thickened and irregular nuclear membranes and may appear pale but upon close inspection, coarse chromatin and prominent nucleoli are present.
Squamous Cell Carcinoma

- Single malignant cells may display dense cytoplasm with possible orangeophilic staining. Nuclear detail is apparent with parachromatin clearing, prominent nucleoli, and irregularities in the nuclear membrane. It is essential to incorporate all criteria for an accurate interpretation.
Squamous Cell Carcinoma

- Isolated abnormal cell forms may be present, intermixed with necrosis in the background. Caudate or elongate cells are maintained during the dispersion process.
Tumor Diathesis — Look-Alikes

- Cytolysis — stripped nuclei and cytoplasmic debris are present.
- Blood — lysed in the background with RBC ghost cells also evident.
- Trich — busy looking slide with organisms and bacteria present.
- Tumor Diathesis — debris tends to clump together with a frayed edge. Malignant cells should also be in evidence.
Look-Alike: Non-Keratinizing Squamous Cell Carcinoma vs. Adenocarcinoma

- Left image: Non-keratinizing squamous cell carcinoma presents predominantly with malignant cells singly and in disorganized, irregular groupings. The abnormal cells around the edge of this group exhibit irregular, hyperchromatic nuclei with coarse chromatin.

- Right image: Adenocarcinomas typically form 3 dimensional cell clusters and may exhibit a “rounder” presentation due to the liquid based fixation. The nuclei may scallop around the outside edge of the group and display finely granular chromatin and prominent nucleoli. Cytoplasmic vacuoles may be seen.
Glandular Cell Abnormalities

• Revised Bethesda terminology for reporting abnormal glandular cells.
Epithelial Cell Abnormalities - Glandular

Endocervical adenocarcinoma in situ (AIS)

- Abnormal Architectural Patterns Maintained
- Nuclear Crowding With Hyperchromasia
- Pseudostratification, “Feathering” Identified
- Rosettes & Strips
- Nucleoli Often Present

AIS

Same features as CP may be used for TP.

- Abnormal architectural patterns are maintained.
- Nuclear crowding with hyperchromasia.
- Pseudostratification and “feathering” are identified.
- Cell groups may present in rosettes and strips.
- Nucleoli may be present.
AIS

- At screening power, numerous dark groups of cells should alert you to a possible abnormal process.
AIS

• The tissue pattern of AIS is maintained with nuclear stratification and crowding, with nuclei falling outside of the grouping.
AIS

- This group of glandular cells exhibits nuclear crowding as well as hyperchromatic nuclei with a powdery chromatin pattern. Cell dyshesion is evident along the edges.
**AIS**

- This small group of cells shows pseudostratification with crowded, hyperchromatic nuclei.
AIS

- In AIS nucleoli may be present.
Look-Alike: AIS vs. SIL in Glands

- Left image: AIS exhibits groups with a greater depth-of-focus and nuclear crowding with finely granular or vacuolated cytoplasm.
- Right image: SIL appears flatter with a more sheet-like arrangement. The cytoplasm is dense and the cells can have a lower N/C ratio.
Epithelial Cell Abnormalities - Glandular

**Adenocarcinoma**
- 3D Cell Clusters Maintained
- Well Preserved Nuclear Features
- Tumor Diathesis May Be Noted

**Adenocarcinoma**
- 3D cell clusters maintained.
- Well preserved nuclear features are evident.
- Tumor diathesis may be noted with invasive glandular lesions.
Endocervical Adenocarcinoma

- Cluster of abnormal glandular cells with scalloped edges and irregular nuclei with finely granular chromatin and nucleoli.
Endocervical Adenocarcinoma

- Loose cluster of large malignant cells exhibiting “rounding up.” Also present are smooth cytoplasmic borders and nuclei with irregular nuclear membranes and irregular distribution of chromatin.
Endocervical Adenocarcinoma

- Loose cluster of malignant cells with vacuolated cytoplasm and eccentric irregular nuclei.
Endometrial Adenocarcinoma

- Papillary grouping with increased depth-of-focus and poly engulfment.
- High N/C ratio and eccentric nuclei with nucleoli.
Endometrial Adenocarcinoma

- Papillary cluster of uniform, bland appearing malignant epithelial cells.
Endometrial Adenocarcinoma

- Dense cluster of malignant cells with scalloped edges, vacuolated cytoplasm, enlarged irregular nuclei with nucleoli and engulfed polys.
Small Cell Undifferentiated Carcinoma

- Aggregates of small cells with hyperchromatic nuclei and nuclear molding. Note the size compared to the neutrophil.
Malignant Mixed Mesodermal Tumor

A: Adenocarcinoma component – a 3 dimensional cluster of tightly packed cells showing poly engulfment.

B: Malignant spindle cell component – a loose aggregate of spindled tumor cells with irregular nuclei and nucleoli. This component can be of either squamous or mesenchymal origin.
Malignant Melanoma, metastatic to cervix

- Numerous single malignant cells with irregular nuclei, prominent nucleoli and some evidence of brown pigment in the cytoplasm.
Malignant lymphoma, metastatic to cervix

- Necrotic debris with numerous single cells. On high power, nuclear variability is evident and many nuclei appear with clefts and nucleoli.
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