



# Anal Cytology

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## AGENDA

- **OVERVIEW**
- **ANATOMY/HISTOLOGY**
- **COLLECTION**
- **SPECIMEN PREPARATION**
- **CYTOLOGY**

## ANAL CANCER OVERVIEW

Rare in general population, but high and growing in at-risk populations<sup>1</sup>

- Men who have sex with men (HIV+/-)
- Women (HIV)

### Incidence Rates<sup>2,3</sup>

- Men who have sex with men (MSM)
  - HIV- 35/100,000
  - HIV+ est 70/100,000
- General Population
  - <1/100,000

### Anal Cancer 2010 cases<sup>4</sup>

	Cases	Deaths
Men	2,000	280
Women	3,260	440
<b>Total</b>	<b>5,260</b>	<b>720</b>

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<sup>1</sup> Bean, SM, Chhieng, DC, Anal-Rectal Cytology: A Review. *Diagnostic Cytopathology* 2009; Vol 38 No 7, 538-546

<sup>2</sup> Palefsky, J. Screening for Anal and Cervical Dysplasia in HIV-Infected Patients. *The PRN Notebook*. Volume 6, No. 3, Sept. 2001. 24-31.

<sup>3</sup> Darragh, TM. Anal Cytology for Anal Cancer Screening: Is it Time Yet? *Diagnostic Cytopathology*, 2004; Vol 30, No 6, 371-374

<sup>4</sup> American Cancer Society, Cancer Facts and Figures, 2010

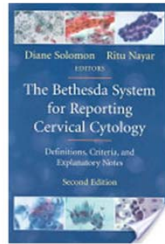
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Anal cancer is rare in the general population but high and growing in at-risk populations which are men who have sex with men regardless of HIV status. Women are considered at risk when they are HIV+, have a history of cervical dysplasia, vulvar cancer, or anal condyloma. Incidence rates as stated on the slide.

## ANAL CANCER OVERVIEW

- Morphologic & biologic similarities between anal intraepithelial neoplasia (AIN) and cervical intraepithelial neoplasia (CIN)<sup>1</sup>
- Association with sexual transmission of oncogenic HPV, especially type 16<sup>1</sup>
- Gardasil® HPV-vaccine approved to prevent anal cancer<sup>2</sup>



*2001 Bethesda guidelines includes appendix for anal cytology*

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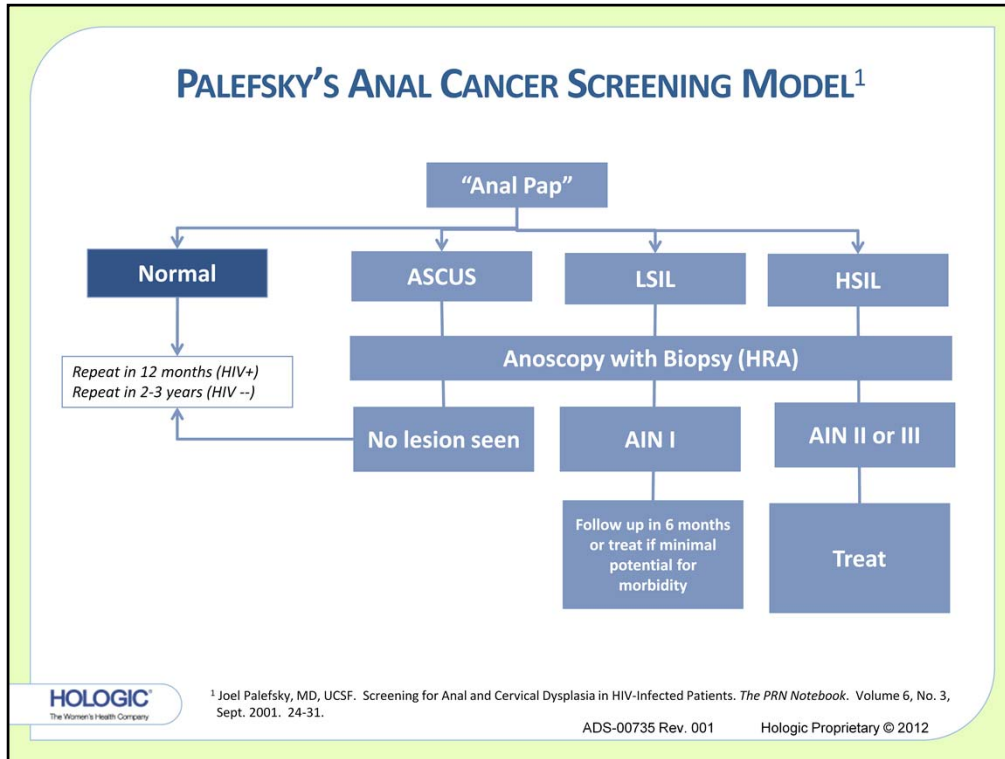
<sup>1</sup> Darragh, TM. Anal Cytology for Anal Cancer Screening: Is it Time Yet? Diagnostic Cytopathology, 2004; Vol 30, No 6, 371-374

<sup>2</sup> FDA News Release, Dec 22, 2010 (<http://www.fda.gov/newsevents/newsroom/pressannouncements/ucm237941.htm>)

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There are morphologic and biological similarities between AIN and CIN. There is a causal relationship link between high risk HPV and the development of anal carcinoma. HPV types 16/18 account for 85% of the cases. Gardasil vaccine has been approved to prevent anal cancer. There are no FDA approved test for anal HPV testing; however, some labs offer this type of testing off-label. The 2001 Bethesda guidelines includes an appendix for anal cytology.



Dr. Joel Palefsky at UCSF has proposed an anal cancer screening model.

## ANATOMY OF ANAL CANAL

- 3- 4 cm long tubular structure<sup>1</sup>
- Surrounded by smooth muscle
- From anal verge to rectal mucosa
- Delineated by anal-rectal transformation zone

*Samples should be from entire anal canal including keratinized and nonkeratinized squamous epithelium and anorectal transformation zone<sup>2</sup>*

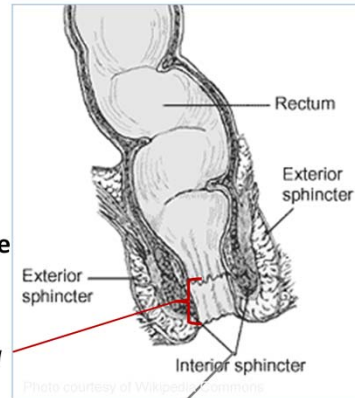


Photo courtesy of Wikipedia Commons

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<sup>1</sup>Lawson, J.O. Pelvic anatomy. II. Anal canal and associated sphincters. Ann R Coll Surg Engl. 1974 June; 54(6): 288-300.  
<sup>2</sup>Bean, SM, Chhieng, DC, Anal-Rectal Cytology: A Review. Diagnostic Cytopathology 2009; Vol 38 No 7, 538-546

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The anal canal is a 3-4 cm long tubular structure which is surrounded by smooth muscle. The canal extends from the anal verge to the rectal mucosa and is delineated by the anal-rectal transformation zone. Samples should be taken from the entire canal to ensure proper sampling.

## SPECIMEN COLLECTION<sup>1</sup>

- **Patient positioning**
  - Lateral recumbent
  - Dorsal lithotomy in women
- **Cytobrush or Dacron Swab**
- **Blindly without proctoscope or anoscope or with high resolution anoscopy**

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<sup>1</sup>Bean, SM, Chhieng, DC, Anal-Rectal Cytology: A Review. Diagnostic Cytopathology 2009; Vol 38 No 7, 538-546

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The patient may be positioned either dorsal lithotomy position (The dorsal (or supine) position means to lie on one's back. The lithotomy position is where the patient has his/her feet elevated above the hips and sometimes above the head depending on the procedure, in stirrups. This is the most common position for childbirth and pelvic exams. ) or lateral recumbent. (the posture assumed by the patient lying on the left side with the right thigh and knee drawn up)

Specimens may be collected “blind” without the aid of proctoscope or anoscope. Specimens are often collected under high resolution anoscopy ( this type of collection requires that the collector is certified in the use of this instrument).

LABORATORY SOLUTIONS

## Quick Reference Guide Anal Cytology Specimen Collection



Collect...



Rinse...



Tighten...



Record...



Place...

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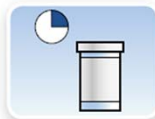
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## SPECIMEN PREPARATION

- **Blue filter**
- **ThinPrep® 2000:** Sequence 2
- **ThinPrep® 5000:** Non-Gyn Sequence



**Let stand for  
15 minutes**



**Gently shake**



**Run, Stain,  
Evaluate**

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The lab should prepare the specimen on the TP Processor using a Blue filter, using sequence 2 on the TP 2000 or the Non Gyn sequence on the TP 5000.

## SPECIMEN ADEQUACY

### The Bethesda System 2001

- 2,000 – 3,000 nucleated squamous cells
- ThinPrep® : 1 – 2 nucleated squamous cells per high power field

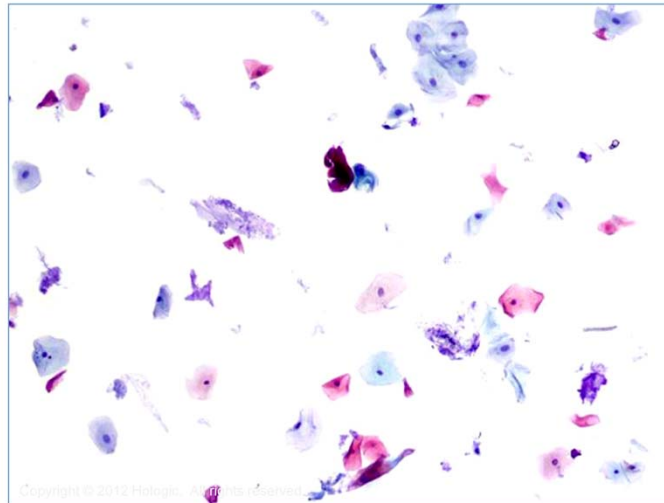
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According to Bethesda 2001 for an anal pap to be considered satisfactory it should contain 2,000-3,000 nucleated squamous cells. For the Thin Prep specimen this would equate to 1-2 nucleated squamous cells per high power field.

## ADEQUATE SPECIMEN



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This field of view would demonstrate an adequate specimen showing numerous nucleated squamous cells along with expected background mucous and anucleated squames.

## SPECIMEN ADEQUACY

### **Transformation zone component**

- Glandular epithelium
- Not required for satisfactory specimen
- Presence or absence should be noted

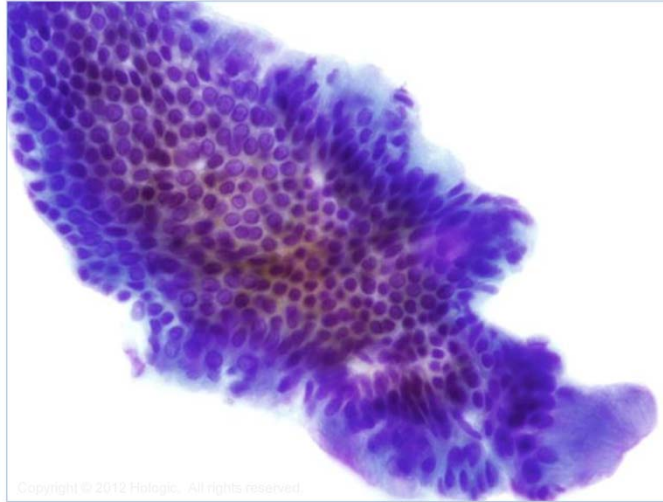
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Transformation zone component (glandular epithelium) is not required for a satisfactory specimen; however the presence or absence of transformation zone component should be noted in the report.

## GLANDULAR EPITHELIUM



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Glandular epithelium from the transformation zone note the similarity to endocervical cells. The same criteria for normal glandular epithelium would apply.

## SPECIMEN ADEQUACY

### Obscuring material

- Fecal material
- Bacteria
- Inflammation
- Mucus
- Blood

*May hinder microscopic evaluation*

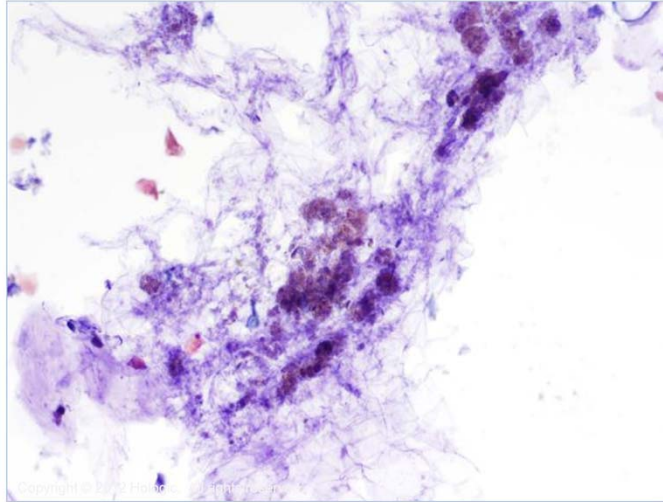
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Frequently obscuring material may be observed in anal paps. This obscuring material would include fecal material, bacteria, inflammation, mucus, and blood. All of these materials may hinder microscopic evaluation just as in other cytologic samples.

## FECAL MATERIAL



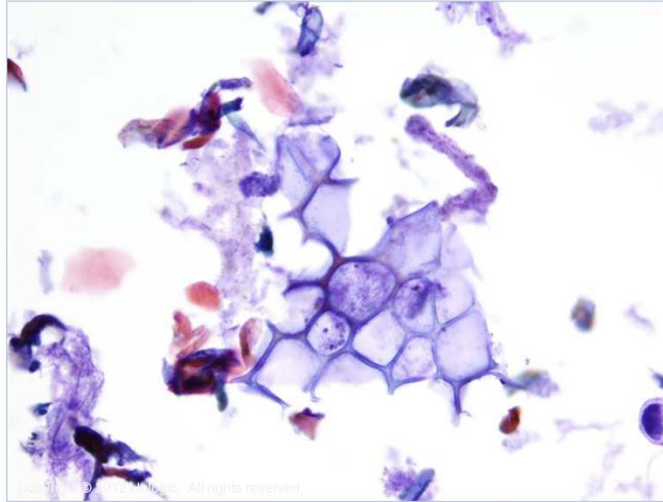
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10x Fecal Material along with anucleated squames

## FOOD MATERIAL



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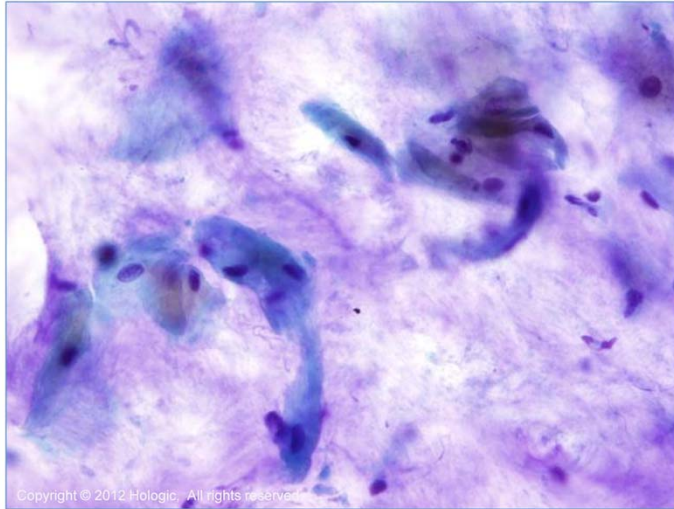
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20x Vegetable or food material along with anucleated squames



## Mucus



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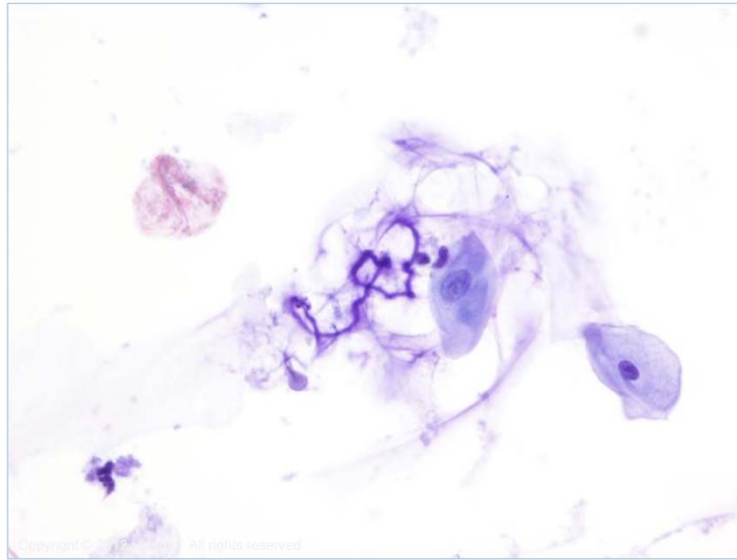
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Mucus present obscuring nucleated squamous cells.

## CURSCHMANN'S SPIRAL

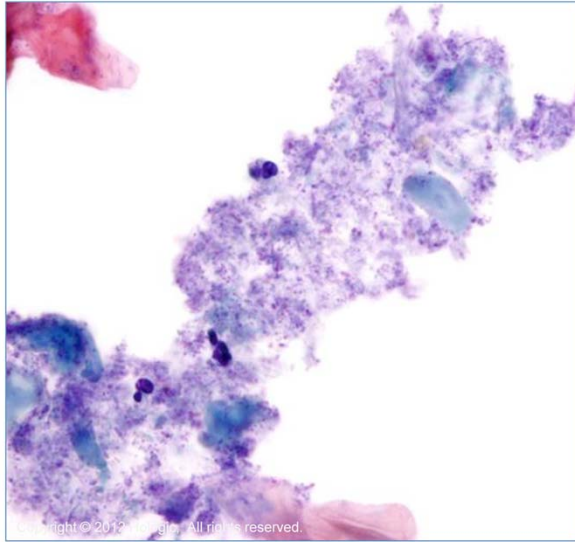


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Curschmann's Spiral present in mucus along with nucleated squamous cells

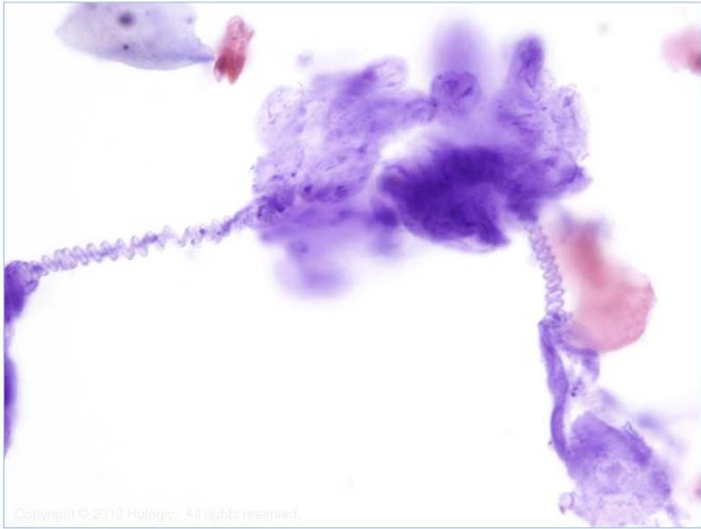
## BACTERIA



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Bacteria present obscuring cellular material

# DACRON FIBER



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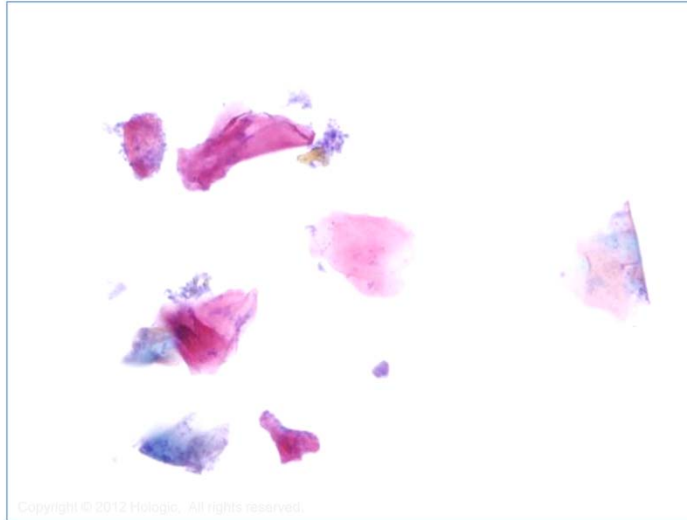
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## SPECIMEN ADEQUACY

### Unsatisfactory Specimen

- If predominately anucleated squames
- Mostly obscured by:
  - Mucus
  - Bacteria
  - Fecal material

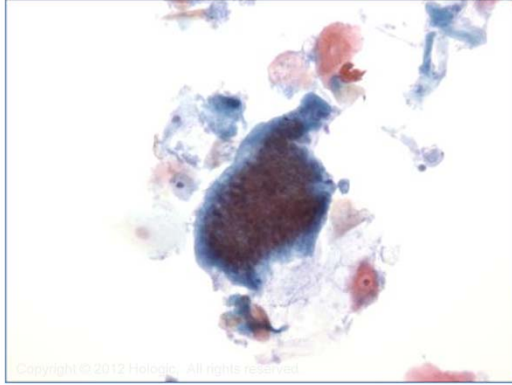
## ANUCLEATED SQUAMES



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## NORMAL CYTOLOGY



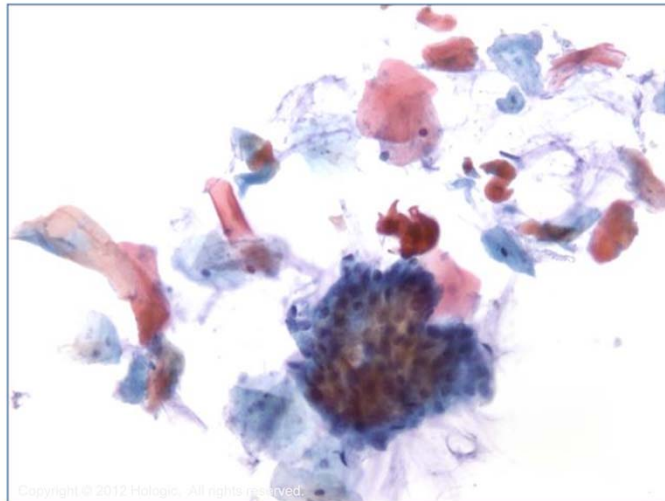
- Nucleated squamous cells
- Glandular epithelium
- Anucleated squames
- Fecal contaminant

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## NORMAL CYTOLOGY



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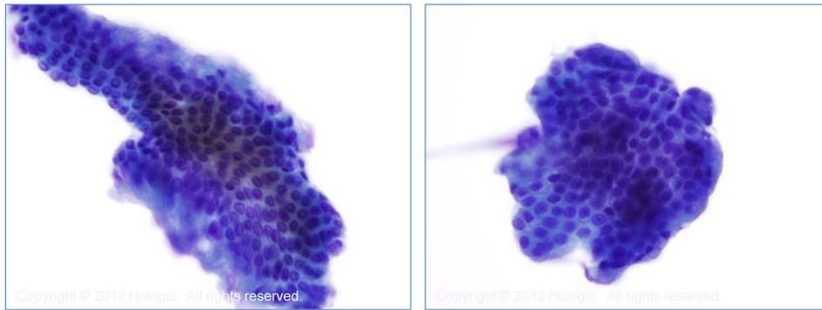
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This field of view show anucleated and nucleated squamous cells, mucus, and glandular cells



## NORMAL CYTOLOGY

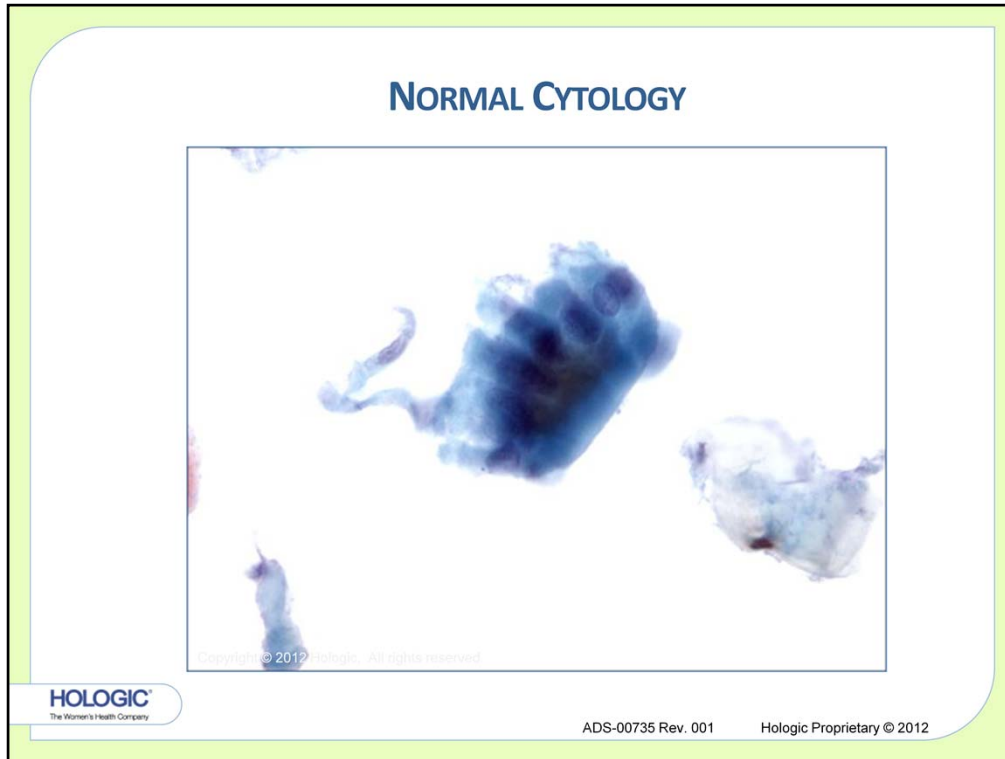


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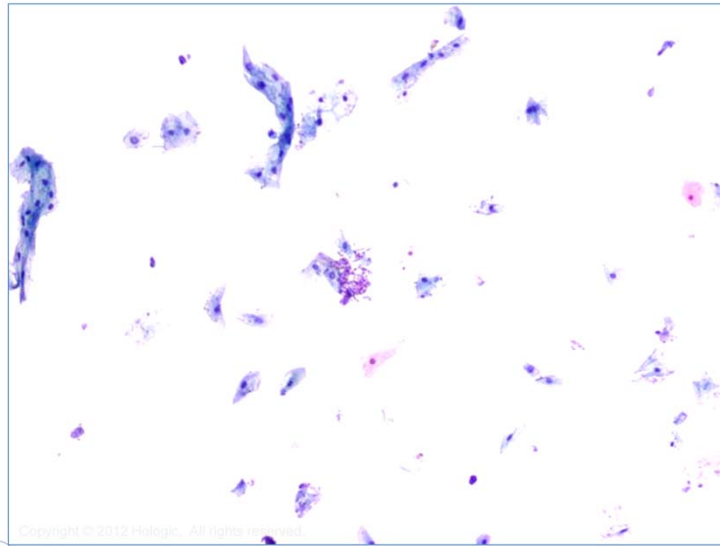
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60x Cells representing normal features of glandular epithelium. Individual, regular nuclei



40x Glandular epithelium in columnar picketed fence appearance

## FUNGAL SPORES



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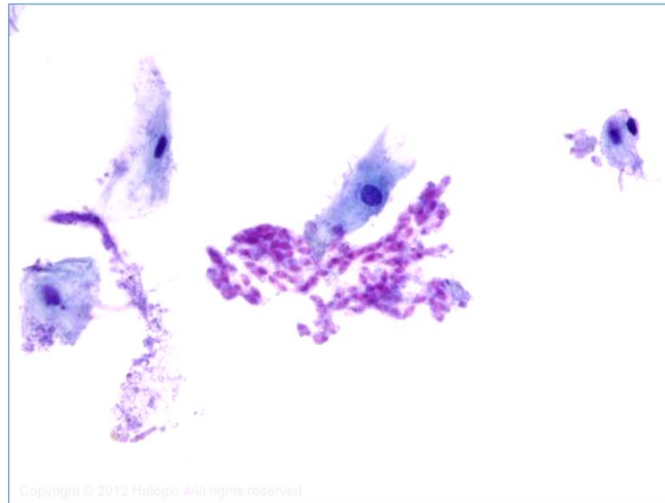
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10x Fungal spores present along with normal squamous epithelium

## FUNGAL SPORES



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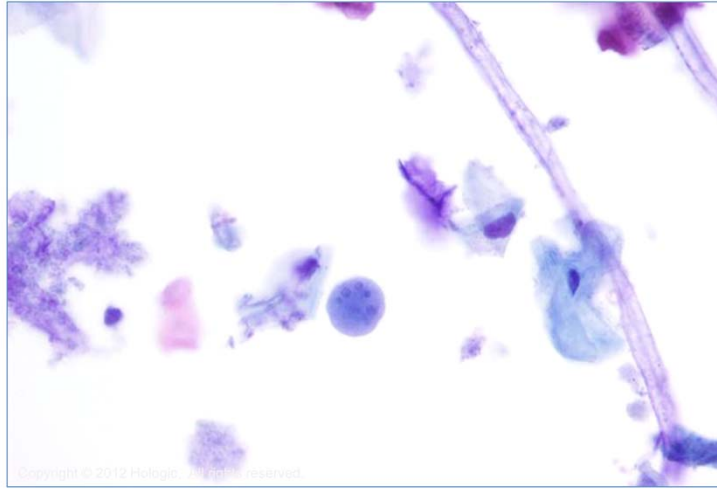
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40x High power view of fungal spores and normal squamous cells.

## AMEBIC CYST



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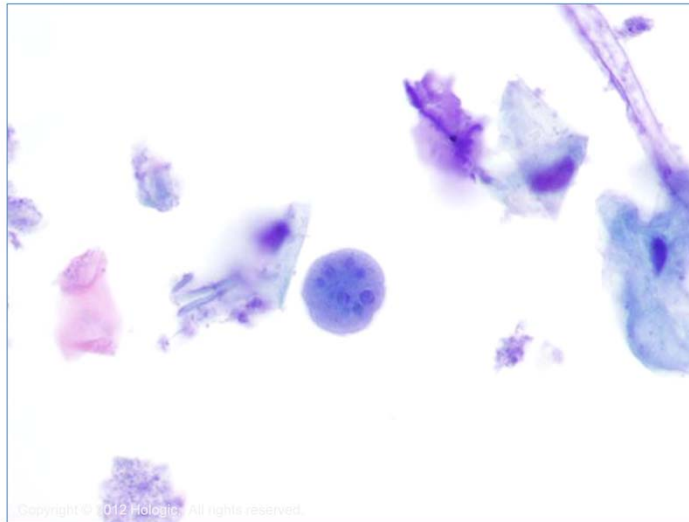
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40x

## AMEBIC CYST



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60x

## ASCARIS OVA



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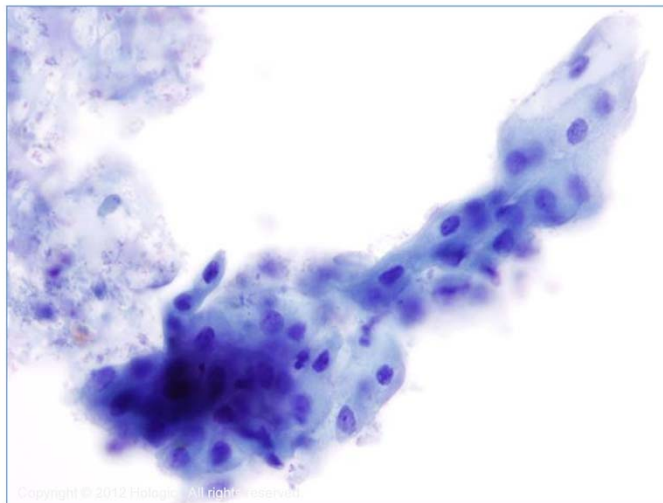
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40x

## REACTIVE SQUAMOUS CELLS



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40x



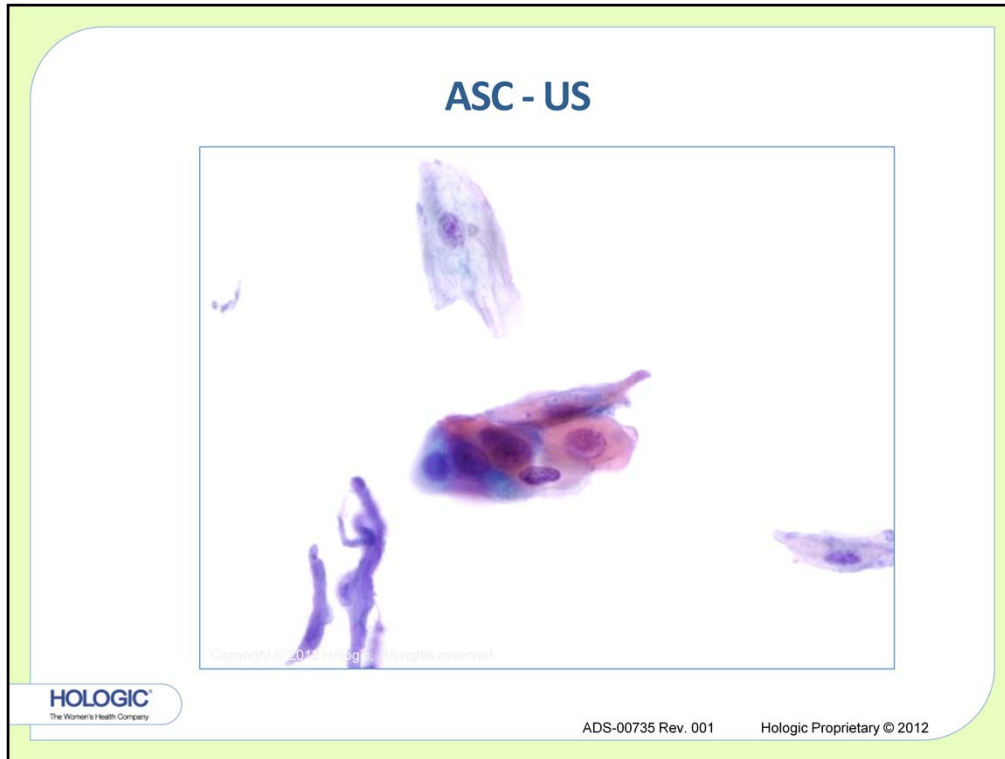
## ATYPICAL SQUAMOUS CELLS UNDETERMINED SIGNIFICANCE

- Cells are found in sheets or singly
- Nuclei  $2^{1/2}$  – 3 times the size of an intermediate nucleus
- Uniform chromatin distribution



40X: Atypical cells of undetermined significance. Atypical mature squamous cells that do not meet the criteria required for an interpretation of low-grade intraepithelial lesion. (LSIL)  
Specific criteria similar to cervical cytology:

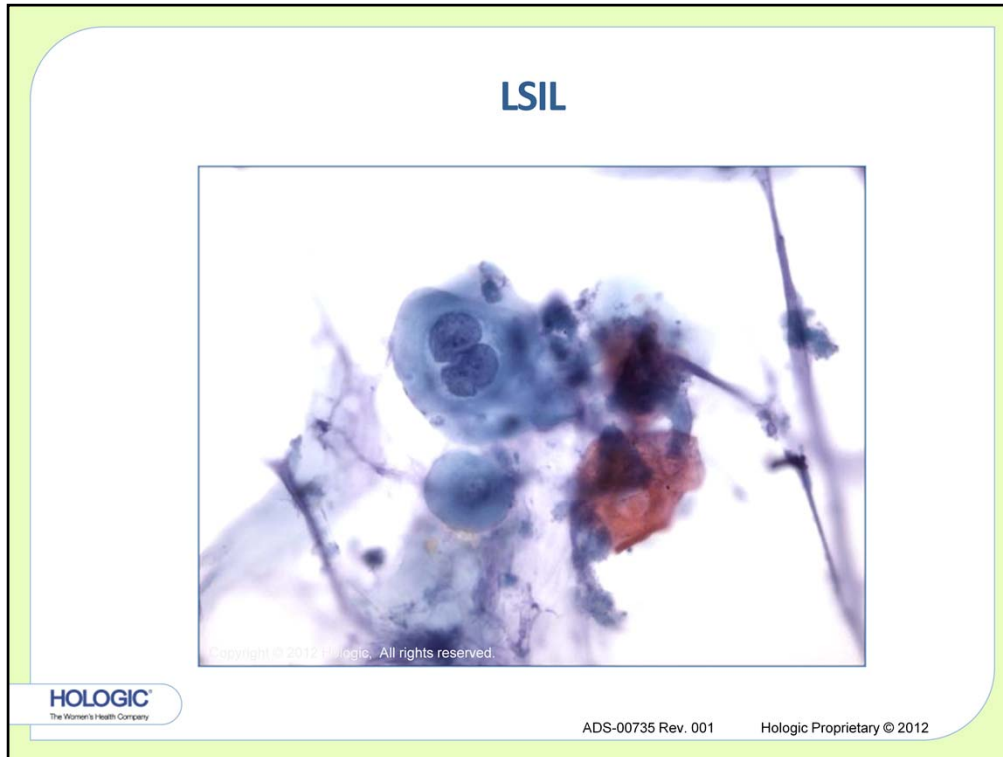
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40X: Atypical cells of undetermined significance. Atypical mature squamous cells that do not meet the criteria required for an interpretation of low-grade intraepithelial lesion. (LSIL)

## LOW GRADE SIL

- Increased Nuclear Detail
- Irregular Nuclear Membrane
- Nuclei 3-4X Intermediate Nucleus
- Sharp, Irregular Cytoplasmic Cavitation (HPV Effect)
- Cytoplasmic Keratinization more prominent than in cervical squamous lesions



40X: Criteria for low-grade intraepithelial lesions (LSIL) are the same as they are for cervical specimens:

- Cells occur singly and in sheets
- Cytologic changes are usually confined to cells with “mature” or superficial-type cytoplasm
- Increased Nuclear Detail
  
- Irregular Nuclear Membrane
  
- Nuclei 3-4X Intermediate Nucleus
- Sharp, Irregular Cytoplasmic Cavitation (HPV Effect)
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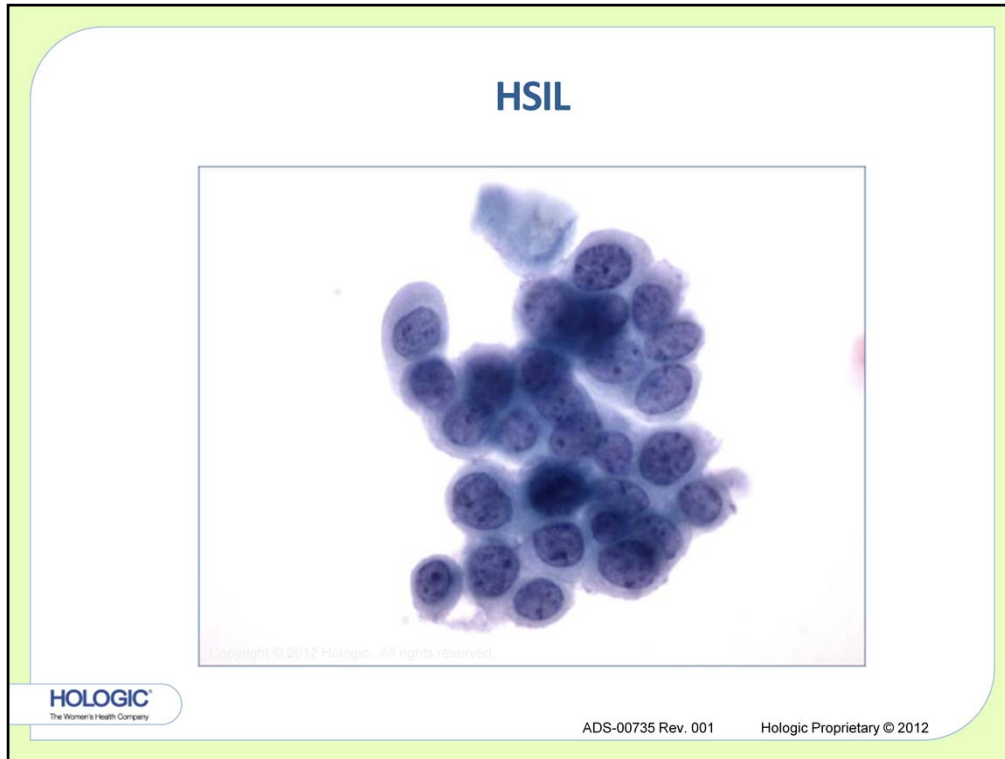
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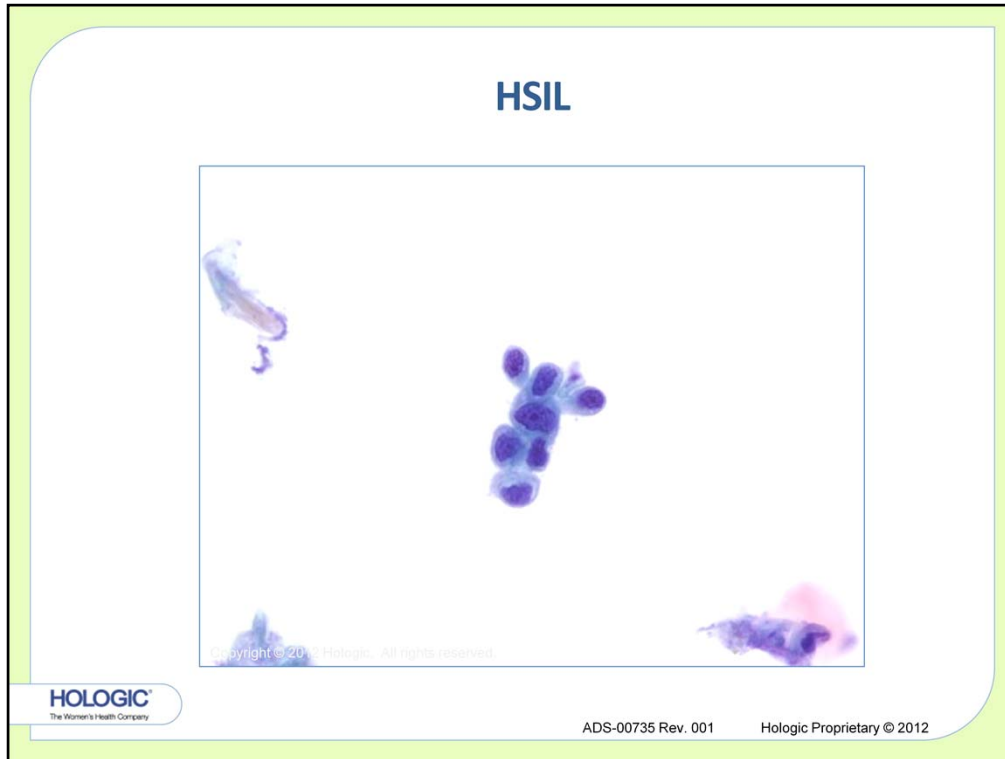
## HIGH GRADE SIL

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

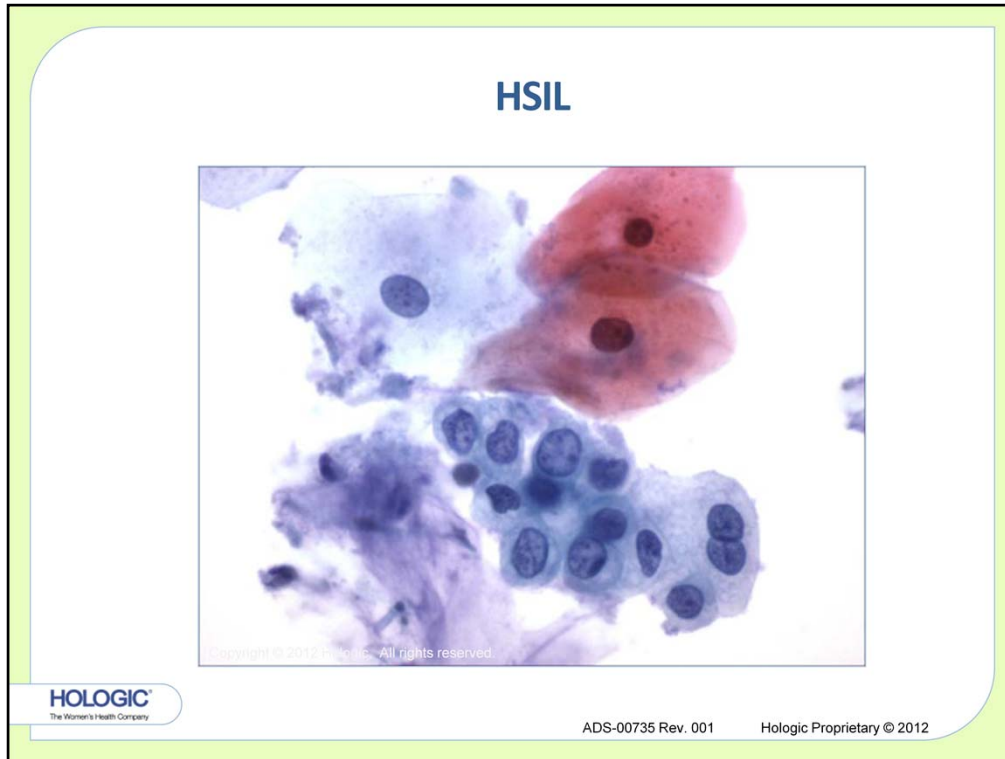




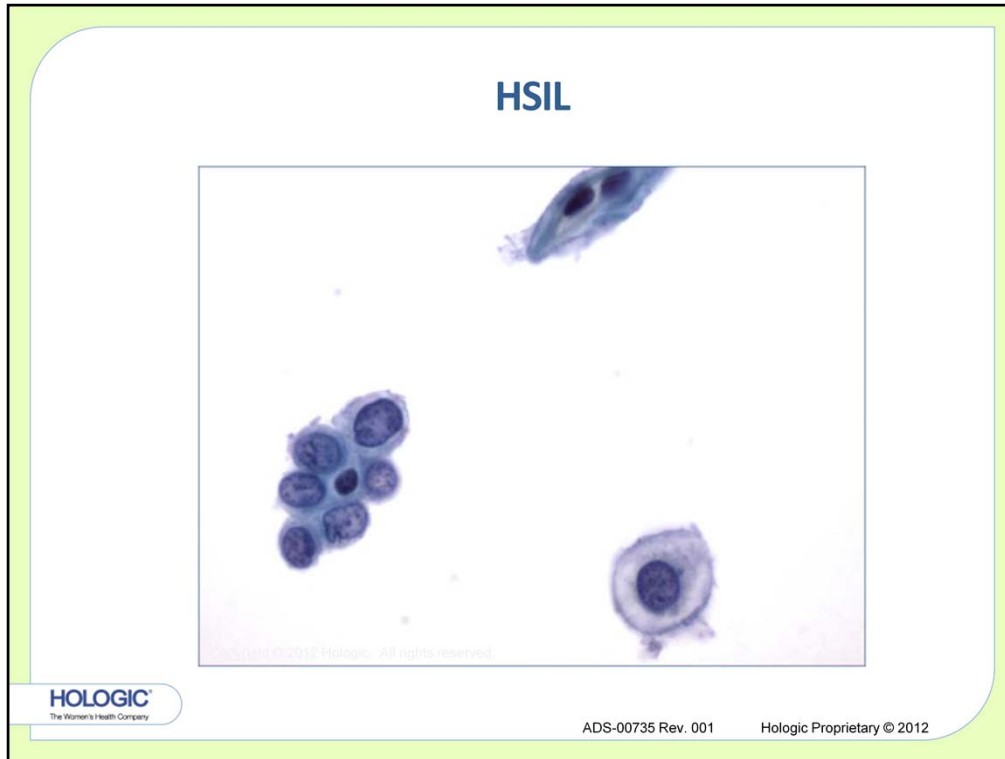
40X: Criteria for high-grade intraepithelial lesions (HSIL) are the same as they are for cervical specimens:



40X: Criteria for high-grade intraepithelial lesions (HSIL) are the same as they are for cervical specimens:



60X: Criteria for high-grade intraepithelial lesions (HSIL) are the same as they are for cervical specimens:

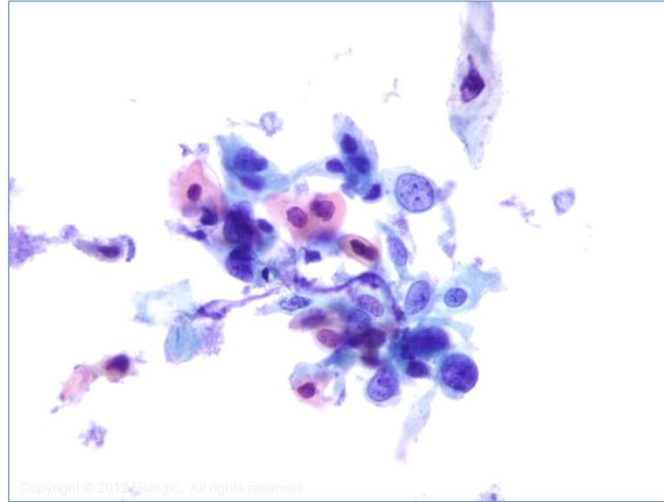


60X: Criteria for high-grade intraepithelial lesions (HSIL) are the same as they are for cervical specimens:

## SQUAMOUS CARCINOMA

- Sheets and single pleomorphic tumor cells
- Hyperchromatic nuclei
- Coarsely granular, unevenly distributed chromatin
- Irregular nuclear contours
- Nucleoli may be present

## POSSIBLE SQUAMOUS CARCINOMA



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40X: squamous carcinoma is difficult to diagnose with cytology alone due to the lack of tumor diathesis which may be due to the fact that the rectum is a closed system which would allow exfoliated material and cellular debris to be excreted with feces.

## CASE STUDY:

- **History: Asymptomatic 35 year old male**
- **Specimen type: Anal cytology**
  - Specimen was collected using Dacron Swab under proctoscopic visualization

This case was provided by Dr. Gabriele Medley, Principal Investigator - "The optimal anal collection technique for screening of anal intraepithelial neoplasia and anal HPV infection", National Centre in HIV Epidemiology and Clinical Research Darlinghurst Australia – Faculty of Medicine UNSW

## CASE STUDY:

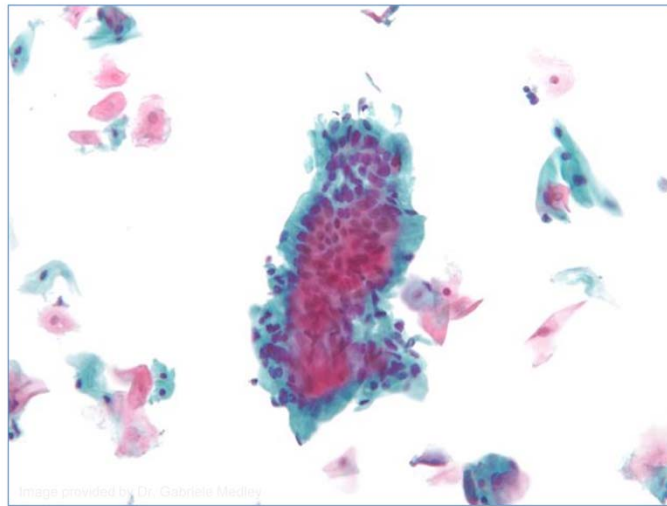


Image provided by Dr. Gabriele Medley

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This case was provided by Dr. Gabriele Medley, Principal Investigator - "The optimal anal collection technique for screening of anal intraepithelial neoplasia and anal HPV infection", National Centre in HIV Epidemiology and Clinical Research Darlinghurst Australia - Faculty of Medicine UNSW

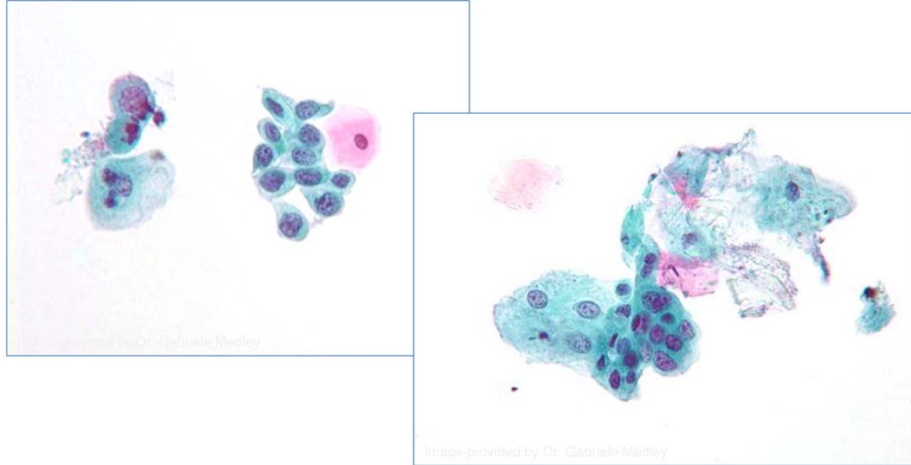
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This 40X image shows a group of benign rectal columnar cells. Notice how closely they resemble the normal endocervical component of the Pap Test.



## CASE STUDY:



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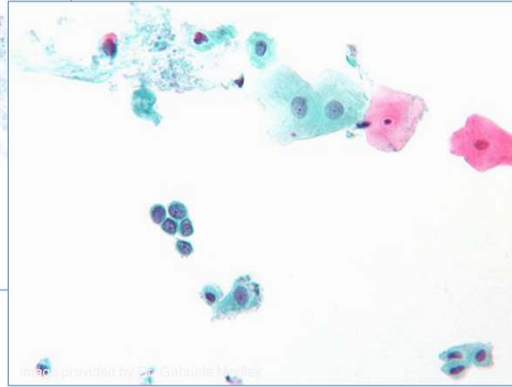
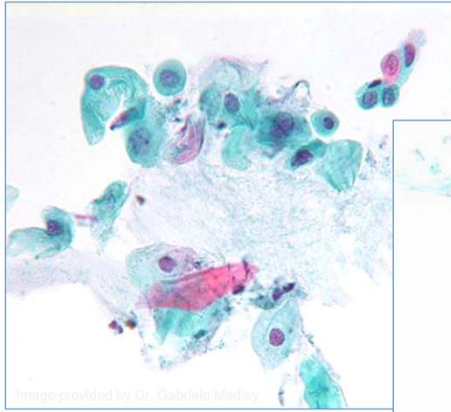
This case was provided by Dr. Gabriele Medley, Principal Investigator - "The optimal anal collection technique for screening of anal intraepithelial neoplasia and anal HPV infection", National Centre in HIV Epidemiology and Clinical Research Darlinghurst Australia - Faculty of Medicine UNSW

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The cells in these images taken at 40x are readily identified as those arising in a high grade lesion. They show high nuclear to cytoplasmic ratios, abnormal chromatin patterns and irregular nuclear membranes.

## CASE STUDY:



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The small immature cells in these two high power views show irregularly distributed chromatin and only a light rim of cytoplasm. They are consistent with a diagnosis of AIN III, severe dysplasia.

## CASE STUDY:

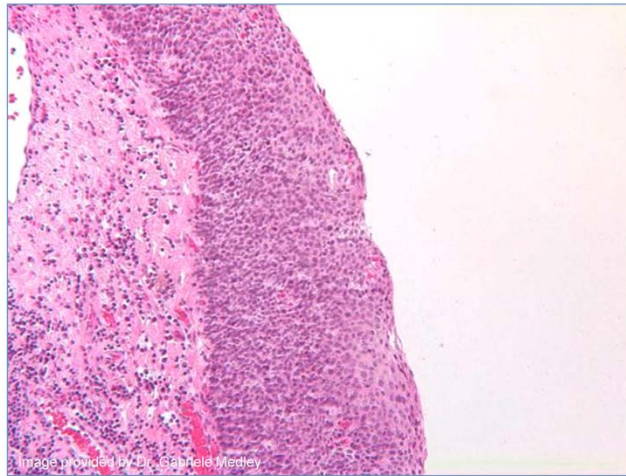


Image provided by Dr. Gabriele Medley

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Histologic section of the lesion at 20x

## CASE STUDY:

- **Cytologic Diagnosis:**
  - High Grade SIL (AIN III)
- **Tissue Diagnosis:**
  - High Grade SIL/AIN III

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## Bibliography

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