Chapter 3
Cervical and Vaginal Cytology

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The Bethesda system provides a uniform reporting system for cervical cytology specimens. Recommended adequacy for liquid-based preparation is at least 5000 squamous cells, and for conventional smears is between 8000 and 12,000 squamous cells [1] (Fig. 3.1).

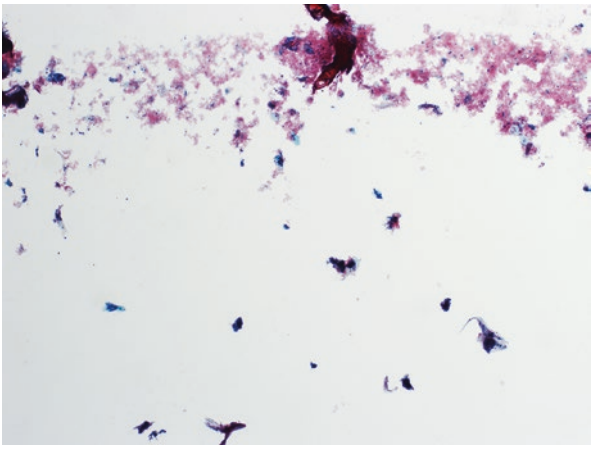

Fig. 3.1 Unsatisfactory for evaluation due to lack of sufficient squamous component (ThinPrep × 40)

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Different Preparations and Screening Procedures [2] (Table 3.1)

Two commonly used techniques for collecting Pap smears are liquid-based and conventional.

- Conventional Smear
  Often obtained using the combination of a spatula and brush.
- Liquid-Based Cytology
  It can provide the opportunity to prepare duplicate slides and cell blocks and the option of additional testing, including HPV, *chlamydia*, and gonorrhea. It is also a substrate for automated screening devices.
  - *ThinPrep* uses a methanol-based preservative solution (PreservCyt Solution). The preparation method involves dispersion, and cell collection, followed by cell transfer to slides.
  - *SurePath* uses an ethanol-based preservative solution. The preparation method involves vortexing, disaggregation, and transfer to a sedimentation tube, and a pallet is obtained, sedimentation by gravity, followed by cell deposition and Papanicolaou staining.

Table 3.1 Cervical cancer screening guidance (American Society for Colposcopy and Cervical Pathology 2021) [3]

1.	In an age group less than 25 years, no screening is suggested
2.	The preferred screening method for age groups between 25 and 65 years is HPV testing alone (using an FDA-approved platform) every 5 years. However, other acceptable options are using the Co-test method (HPV and cytology) every 5 years or every 3 years with cytology alone
3.	In those over 65 years old, no screening is required if prior screening is negative. This negative screening includes two negative primary HPV tests, or two negative co-tests, or three negative cytology alone tests within the last 10 years (with the most recent test within the past 3–5 years)

Normal Elements (Superficial, Intermediate, Parabasal, and Navicular) [1, 4]

- Squamous cells include basal cells, parabasal cells, and intermediate cells.

  Basal cells are usually small cells along the basement membrane and resemble small histocytes, as seen in the atrophic specimen primarily as syncytial aggregates.

  Parabasal cells are the first to acquire squamous features, dense cytoplasm, and distinct cell boundaries. In addition, they have moderately abundant cytoplasm and round to oval nuclei. Parabasal cell predominates in atrophic specimens and can also be seen in postpartum conditions or related to the intake of oral contraception (progesterone).

  Intermediate cells provide essential nuclear size reference (average 35mm^2, the size of a red blood cell). Intermediate cells have abundant and transparent cytoplasm. Any squamous nucleus significantly larger and more hyperchromatic than an intermediate cell is likely a dysplastic cell.

  Superficial cells have pyknotic nuclei and delicate transparent cytoplasm.
- Endocervical cells are cohesive fat sheets or strips of uniform cells, appearing "honeycomb" pattern (opposing view) or "picket fence" pattern (side view). The cell shape (nuclei and cytoplasm) can be tall columnar and may have abundant mucinous cytoplasm.

  Reactive endocervical cells can have significant nuclear enlargement, hyperchromasia, and prominent nucleoli but maintain smooth nuclear membranes and fine chromatin, abundant cytoplasm with distinct cell borders, low nuclear/cytoplasmic ratios, and without crowding or overlapping [5]. In addition, they can show binucleation or multinucleation and should not be confused with herpes virus changes.
- Endometrial cells.

  Exfoliated endometrial cells can be seen if a Pap sample is taken during the first 12 days of the menstrual cycle. Morphologically, they appear as a spherical cluster, small cells with a dark nucleus and scant cytoplasm. They are associated with menstrual cycle changes, endometrial pathology (such as endometritis, endometrial polyps, or neoplasia), and exogenous changes (intrauterine devices, IUDs, and others). Exodus represents a group of endometrial cells with endometrial stroma (darker area) in the center surrounded by endometrial glandular cells (Fig. 3.2). Directly sampled endometrial cells present large and small tissue fragments with a combination of glands and stroma.

  Endometrial cells are needed to be reported in women 45 years of age or older. The differential diagnoses of exfoliated endometrial cells include high-grade intraepithelial lesion (HSIL), squamous cell carcinoma, adenocarcinoma in situ (AIS), endometrial carcinoma and rarely a small cell carcinoma.

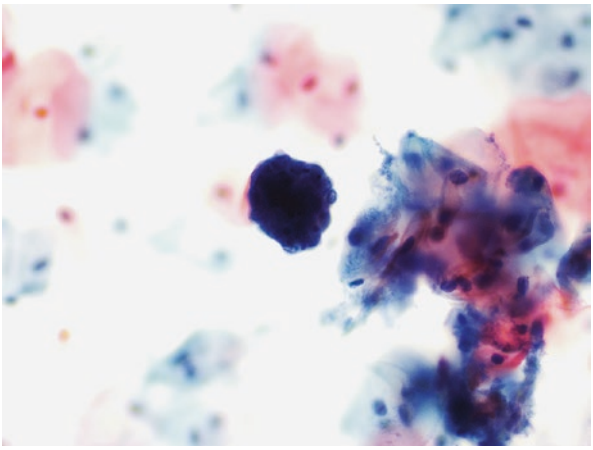

Fig. 3.2 Cluster of endometrial cells with dark central area and lighter periphery (SurePath × 400)

Benign Findings [1, 4] (Table 3.2)

- Parakeratosis is a benign reactive change caused by chronic irritation. It usually shows keratinized squamous cells with dense organophilic cytoplasm and small pyknotic nuclei. However, if nuclear atypia is present, then the term "atypical parakeratosis" is used and categorized as "epithelial cell abnormality" such as ASCUS (atypical squamous intraepithelial lesion) or SIL (squamous intraepithelial lesion), depending on the extent of the abnormality.
- Hyperkeratosis is a benign response of stratified squamous epithelium due to chronic mucosal irritation, as in uterine prolapse. Squamous cells manifested as anucleate, mature, polygonal isolated cells or plaques of tightly adherent isolated cells. Differential diagnosis includes contaminated cells of the skin.
- Atrophic changes happen in late post-menopause (Fig. 3.3), postpartum (Fig. 3.4), lactation, and childhood (except newborn). Atrophic changes can show nuclear enlargement with mild hyperchromasia. However, the nuclear membrane is still uniform, and the chromatin is evenly distributed but often smudgy. There are usually syncytial groups of parabasal cells and may show background inflammation and cell degeneration. A granular basophilic background may be present, and mummified parabasal cells, "blue blobs," can be evident. The differential diagnosis includes endometrial cells, HSIL, or malignancy.
- Pregnancy-related changes include a predominance of intermediate cells and an increase in navicular cells (glycogenated intermediate cells). Other changes and cell types seen in pregnancy are as follows: Syncytiotrophoblastic cells, decidual cells, arias-stella reaction, and cockleburs.

Table 3.2 Differential diagnosis of benign findings in Pap smears

	Differential diagnosis
Atrophy	HSIL Endometrial cells
IUD-related changes	Atypical glandular cells or adenocarcinoma, ASC-H, or HSIL
Pregnancy-related changes	Arias-Stella reaction: Clear cell carcinoma Decidual cells: Squamous intraepithelial lesion (ASCUS or LSIL) Syncytiotrophoblasts: Herpes infection (Fig. 3.11)
Hormone-related changes	Atrophy, HSIL
Repair	Squamous cell carcinoma
Radiation changes	LSIL, malignancy
Endometrial cells	HSIL, atypical glandular cells, or AIS
Lower uterine segment	Atypical glandular cells
Metaplastic cells	ASC-H or HSIL
Glandular cells post hysterectomy	Atypical glandular cells or well-differentiated adenocarcinoma
Follicular cervicitis	Endometrial cells, ASC-H or HSIL, lymphoma

HSIL high-grade squamous intraepithelial lesion, *ASC-H* atypical squamous cells cannot exclude high grade squamous intraepithelial lesion, *IUD* intrauterine device, *LSIL* low-grade squamous intraepithelial lesion

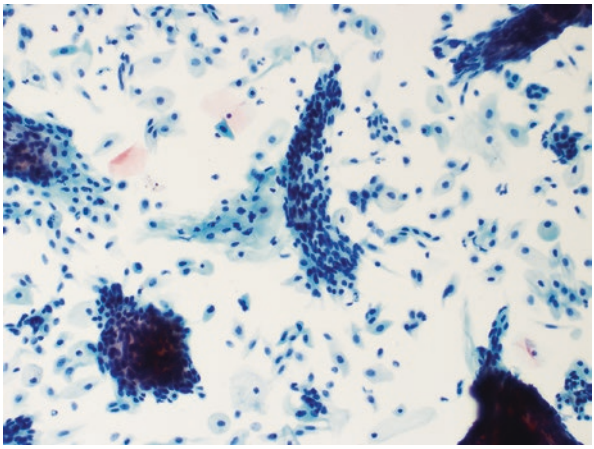

Fig. 3.3 Syncytial groups of parabasal cells suggesting atrophy (SurePath × 200)

- Syncytiotrophoblastic cells are rare findings on Pap smears and may be associated with history of pregnancy. They are large cells with abundant cytoplasm and multiple nuclei.
- Decidual cells are isolated cells with abundant granular cytoplasm, a large vesicular nucleus, and a prominent nucleolus.
- Arias-Stella reaction shows nuclear enlargement, pleomorphism, and hyperchromasia. They have abundant vacuolated cytoplasm without any increase in mitosis and can mimic clear cell carcinoma. It is usually due to hormonal stimulation from pregnancies or trophoblastic disease.

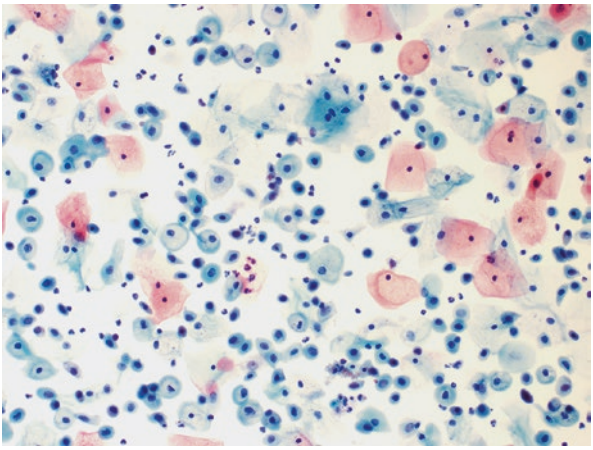

Fig. 3.4 Postpartum changes includes more parabasal cells and less superficial cells (SurePath × 400)

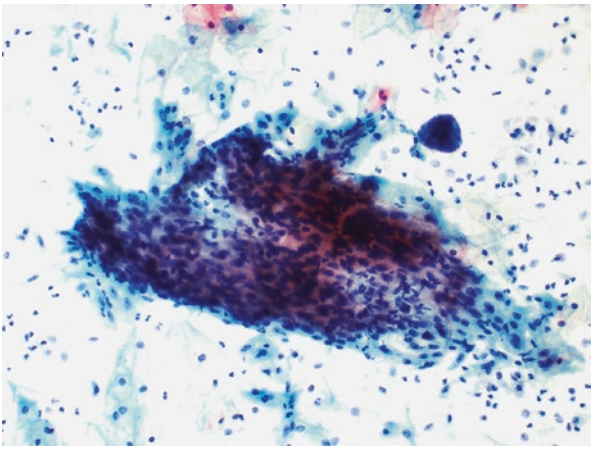

Fig. 3.5 Reactive cellular changes with streaming sheet of cells (ThinPrep × 200)

- Repair
  - Repair results from injury to the cervical epithelium, the proliferation of reserve cells, and reepithelization of ulceration. Cells maintain a uniform polarity that gives the sheets the appearance of streaming or being pulled out, with large nuclei with marked size variation and prominent nucleolus, sometimes irregular in shape (Fig. 3.5). Often, background inflammation can be seen.
- IUD-related changes
  - The characteristic of IUD effect includes the combination of two types of cells a vacuolated cell (Fig. 3.6) and a small dark cell with scant cytoplasm. Sometimes reparative changes are also present, and the background is inflamed. IUD changes may also include reactive changes and inflammation, bacterial vaginosis, and actinomyces [6] (Fig. 3.7). Reactive changes are more prominent in glandular cells and may stimulate an "atypical glandular cells" diagnosis [6].

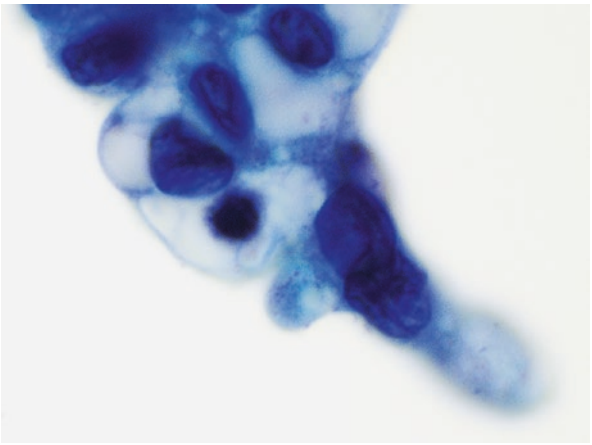

Fig. 3.6 IUD changes include vacuolated cells with large nuclei (ThinPrep × 600)

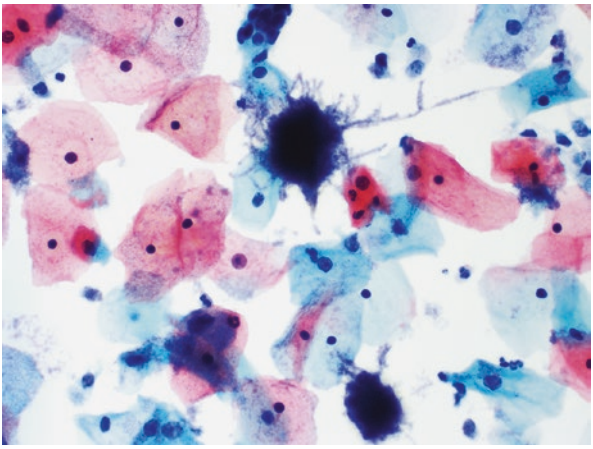

Fig. 3.7 Actinomyces forming a ball-like structure with peripheral filamentous protrusions (SurePath × 400)

- Hormone-related changes
  - The squamous epithelium of the cervix and vagina responds to various stimuli, especially hormones. Estrogen stimulation results in the predominance of a superficial layer. The progesterone effect shows a predominance of intermediate cells. Lack of estrogen effect may result in atrophic pattern smears, which can show a predominance of parabasal cells [7].
- Maturation index: It measures the relationship between the three layers of squamous epithelium (parabasal layer, intermediate cell layer, and superficial layer). The maturation index ratio with the right shift shows an estrogen effect (0/5/95), while the left shift is usually seen in atrophic pattern smears (95/5/0). The mid-zone increase suggests a progesterone effect with an increase in the intermediate cell layer (0/95/5).

- Radiation-related changes
  - Radiation-related changes can show marked cellular and nuclear enlargement with preservation of the nuclear-to-cytoplasmic ratio, abundant cytoplasmic with vacuolization (Fig. 3.8), and cytoplasmic polychromasia.
- Chronic follicular cervicitis
  - This benign reactive condition consists of numerous polymorphous lymphocytes, including occasional plasma cells, indicating follicular cervicitis (lymphoid follicle formation on biopsies).
- Tubal metaplasia
  - Tubal metaplasia is like normal endocervical cells, except for having cilia, and is a benign finding (Fig. 3.9).

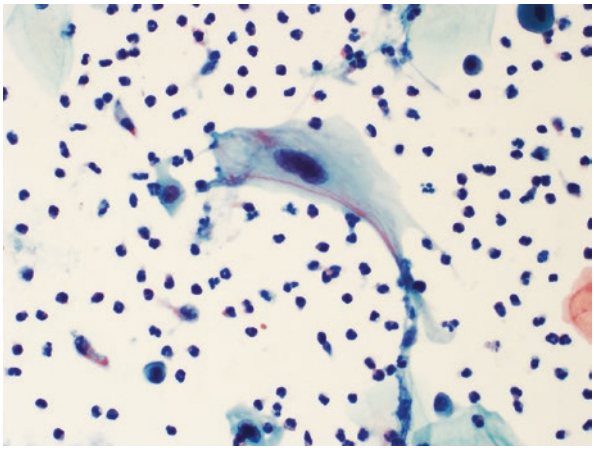

Fig. 3.8 Radiation changes with abundant cytoplasm (SurePath × 400)

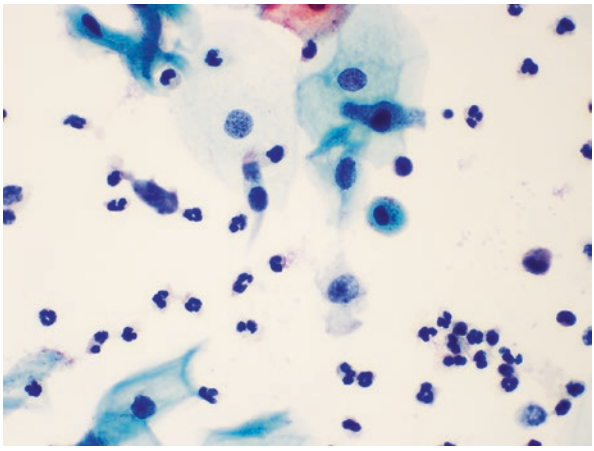

Fig. 3.9 Tubal metaplasia with ciliated epithelial cells (SurePath × 600)

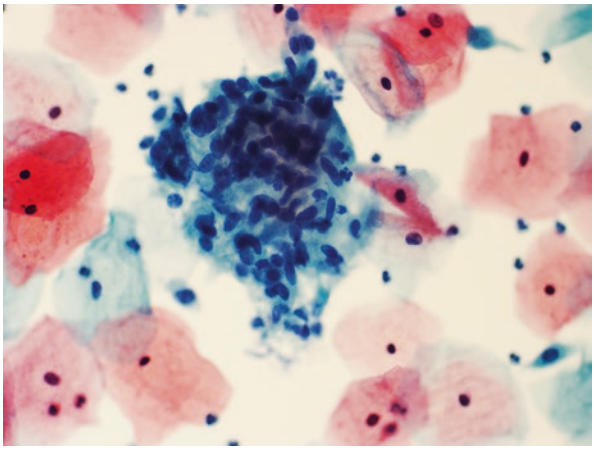

Fig. 3.10 Collection of histiocytes, forming a vaguely formed granuloma (SurePath × 400)

- Glandular cells post-hysterectomy
- Glandular cells post hysterectomy present in a very low subset of vaginal Paps from women who had a total hysterectomy and are benign and usually metaplastic. They resemble normal endocervical cells.
- Other reactive changes:
  - Other reactive changes such as histiocytic collection or granulomatous-type inflammation can be seen (Fig. 3.10).

Organisms and the Pap Test [8]

- Normal Flora and Cytolysis.
  - The vagina epithelium is colonized by Gram-positive, rod-shaped bacteria (*Lactobacillus),* which produce lactic acid, reduce the ambient pH, and may cause cytolysis. *Lactobacillus* can form long rods and mimic fungi or other bacteria.
- Other Organisms and Specific Infections.
  - The common causative viral, bacterial, fungal, and parasite were summarized in Table 3.3.

Table 3.3 Cytomorphologic features, cytomorphologic mimics and molecular studies of common organisms seen on cervical Pap specimens

Microorganisms	Cytomorphology	Differential diagnosis	Ancillary tests
Viruses			
HPV	LSIL: Koilocytosis with perinuclear clearing, nuclear enlargement, nuclear membrane irregularity, hyperchromasia, and binucleation. HSIL: Nuclear membrane irregularity scant cytoplasm, hyperchromasia, nuclear membrane irregularity	Reactive changes or glycogenated cells can mimic LSIL. Parabasal/basal cells may mimic HSIL	Molecular studies: Hybrid capture II assay, Cervista HPV tests, APTIMA HPV assay, Cobas HPV tests DNA and RNA in situ hybridization studies P16 immunostain
HSV	Triad of multinucleation, margination, and molding (Fig. 3.11); background with inflammation and ulceration	Reactive epithelial changes, multinucleated endocervical cells, and radiation changes	PCR on fluids Immunostains for HSV-1 and HSV-2
CMV	Nuclear enlargement, margination, large intranuclear inclusion with a surrounding halo, and small cytoplasmic inclusions	Reactive epithelial changes from ulceration, herpes infection	PCR on fluids; immunostain
Bacteria			
Actinomyces	Gram-positive; thin long filamentous, bacterial forms a ball-like structure with peripheral protrusion of filaments; sulfur granules may be seen	More commonly seen in patients with IUDs *Nocardia* spp., other bacteria or fungi	Not routinely performed
Neisseria gonorrhea [12]	Gram-negative diplococci	Other bacteria	PCR on fluids
Gardnerella vaginalis	A shift in flora: Short bacilli (coccobacilli), curved bacilli, or mixed bacteria; "clue cells" (Fig. 3.12)	Actinomyces	Vaginal pH The amine-odor "whiff" test after addition of potassium hydroxide [KOH]
Leptothrix	Long filamentous bacteria (Fig. 3.13) can be associated with *trichomonas*	Candida	Not routinely performed
Fungal			
Candida	Budding yeast form; may contain true or Pseudohyphae (Fig. 3.14)	Other yeast form fungi	Not routinely performed
Parasite			
Trichomonas vaginalis (Fig. 3.15)	Extracellular flagellated protozoa, pear-shaped; 15–30 μm, red cytoplasmic granules, eccentric nucleus, reactive changes that can mimic koilocytosis	LSIL	*Wet-mount microscopy, rapid antigen-testing* PCR on fluids

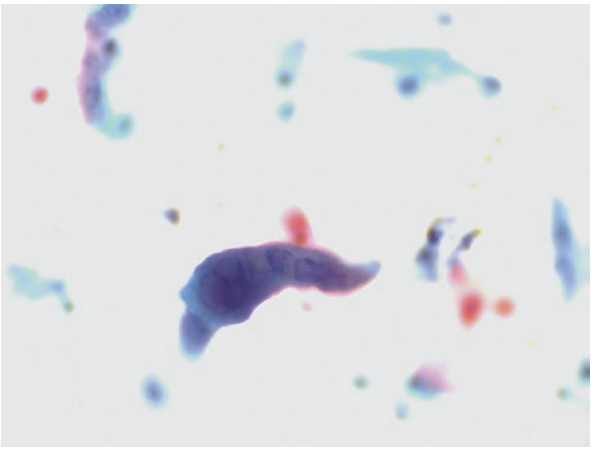

Fig. 3.11 Herpes viral changes with multinucleation, margination, and molding (SurePath × 600)

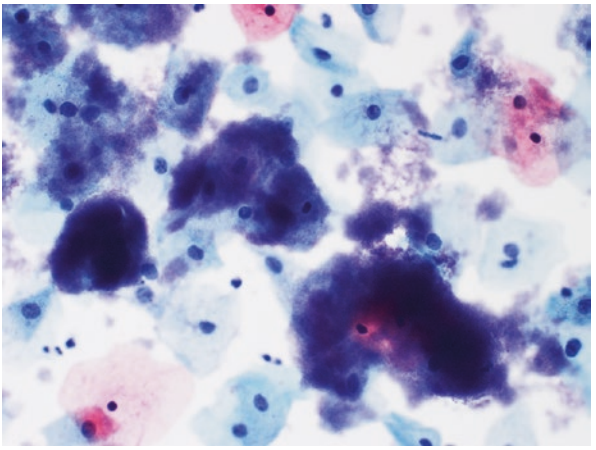

Fig. 3.12 Bacterial colonies on squamous cells "clue cell" (SurePath × 400)

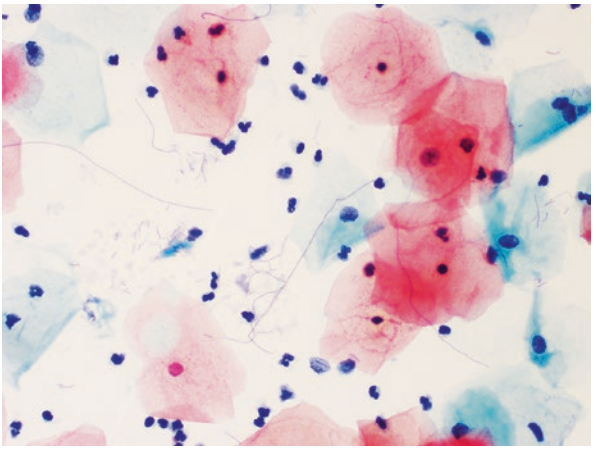

Fig. 3.13 Leptothrix. Long filamentous bacteria (ThinPrep × 200)

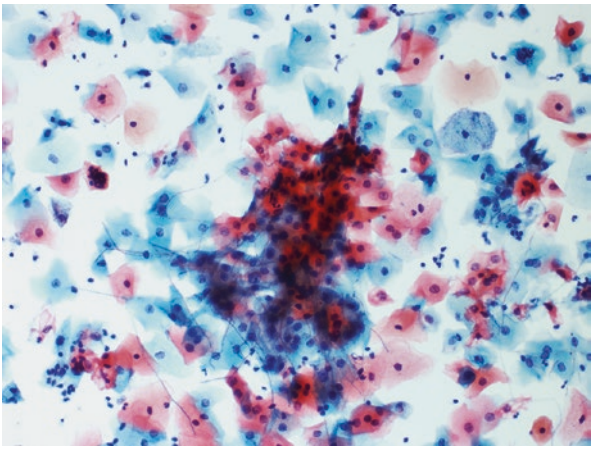

Fig. 3.14 Fungal organisms consistent with Candida (SurePath × 200)

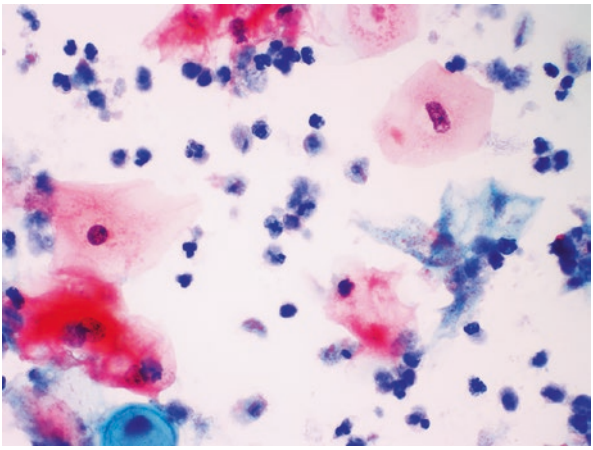

Fig. 3.15 Trichomonas. Pear-shaped organism with red cytoplasmic granules (SurePath × 600)

Ancillary Tests for Detecting Common Organisms in Cervix

- Liquid-based molecular testing for high-risk and low-risk HPV genotype.
- Molecular testing for sexually transmitted diseases (Table 3.3).
  - Human Papilloma virus:

Persistent infection with high-risk human papillomavirus can result in the development of cervical cancer. In addition, genetic and epigenetic alterations in host cell genes are crucial for the progression of cervical precancerous lesions to invasive cancer [9, 10].

More than 100 types of HPV are known, and they are further subdivided into low-risk and high-risk types. Of high-risk types, HPV16 and HPV18 are the most important types and are commonly seen in severe lesions.

According to protein expression during the viral cycle, two functional genome regions have been identified: (1) an early genes region, encoding E1, E2, E4, E5, E6, and E7, and (2) a two late genes region, encoding the major (L1) and minor (L2) capsid proteins [11].

The viral proteins E6 and E7 are overexpressed and block apoptosis and uncouple cell growth arrest by inactivating p53 and pRb, respectively. The inactivation of pRb by E7 forces infected cells to remain proliferative, stimulates the S-phase gene, and fails to stop the cell cycle. This accumulation of DNA damage, centrosome abnormalities, and chromosomal segregation defects eventually leads to genomic instability and carcinogenesis.

Table 3.4 Artifacts, contaminates, and miscellaneous findings features in Pap smears

Findings	Causes/Subcategories	Features
Artifact		
Cornflaking [13]	Air trapped on superficial squamous cells (Fig. 3.16)	Refractile brown or black artifact which obscures nuclear details
Cockleburs [14]	Can be seen in pregnancy but also without pregnancy such as in women with IUDs	Refractile crystalline structures surrounded by inflammatory cells/ histocytes (Fig. 3.17)
Contaminates		
Airborne fungal spores [15]		
	Alternaria	Brown pigmented structure
	Aspergillus	Characteristic "fruiting body," septate hyphae with acute angle branching
Pollen grains [16]	Microscopic masculine cells that come from flower-bearing plants	Mono or multilayer cell wall May be mistaken for protozoa cysts or SIL
Vegetable cells [16] (Fig. 3.18)		May be mistaken for abnormal cells or certain microorganisms
Miscellaneous findings		
Psammoma bodies [17]	Associate with a benign condition in more than half of cases. Clinical and imaging correlation to search for a neoplastic process (such as *Mullerian tumors*)	Concentrically laminated calcifications (Fig. 3.19)
Collagen balls	Commonly seen in serous body cavity fluid but rarely reported in Pap [18]	Translucent blue-green, spheroid hyalinized collagen bodies
Hematoidin crystals [19]	Can be seen in pregnancy or postpartum conditions	Needle shaped brown crystalline material forming rosette-like structure
Blue blobs [20]	Condensed mucus-like material or degenerated nuclei (cyanophilic bodies), seen in some atrophic smears. Should not be interpreted as significant abnormality	Calcifications, HSIL

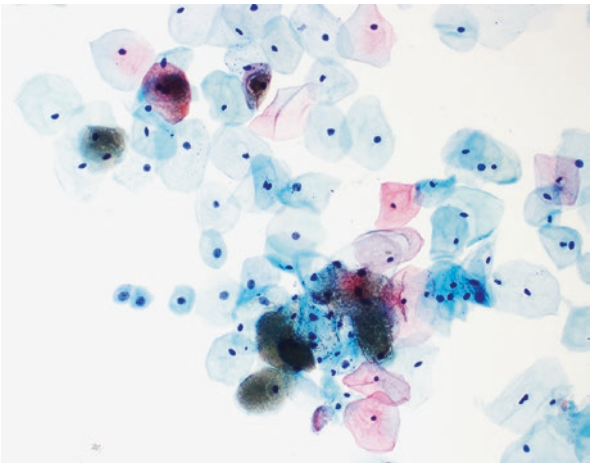

Fig. 3.16 Cornflaking. Brown refractile artifact due to air trapping (ThinPrep × 200)

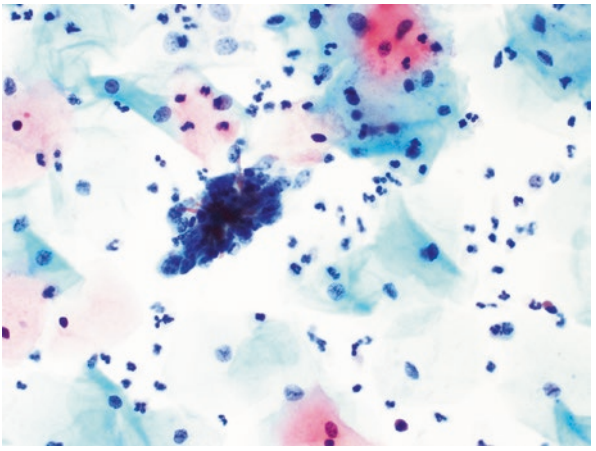

Fig. 3.17 Cockleburs. Refractile crystalline structures surrounded by histocytes (SurePath × 400)

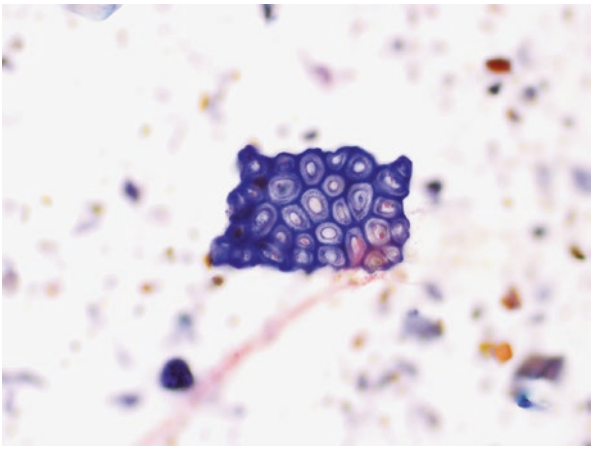

Fig. 3.18 Vegetable material (SurePath × 400)

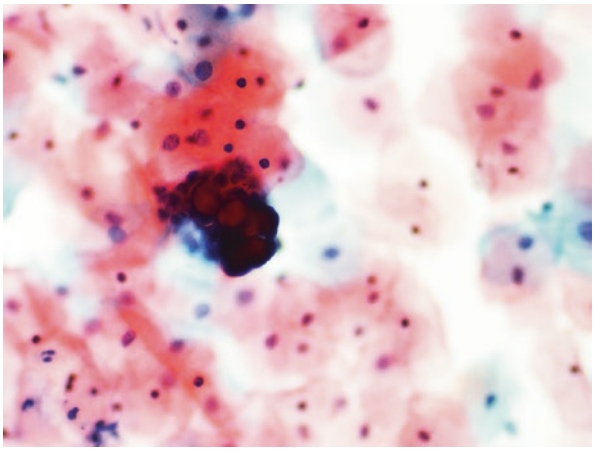

Fig. 3.19 Psammoma calcifications (SurePath × 400)

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Conflict of Interest None.

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