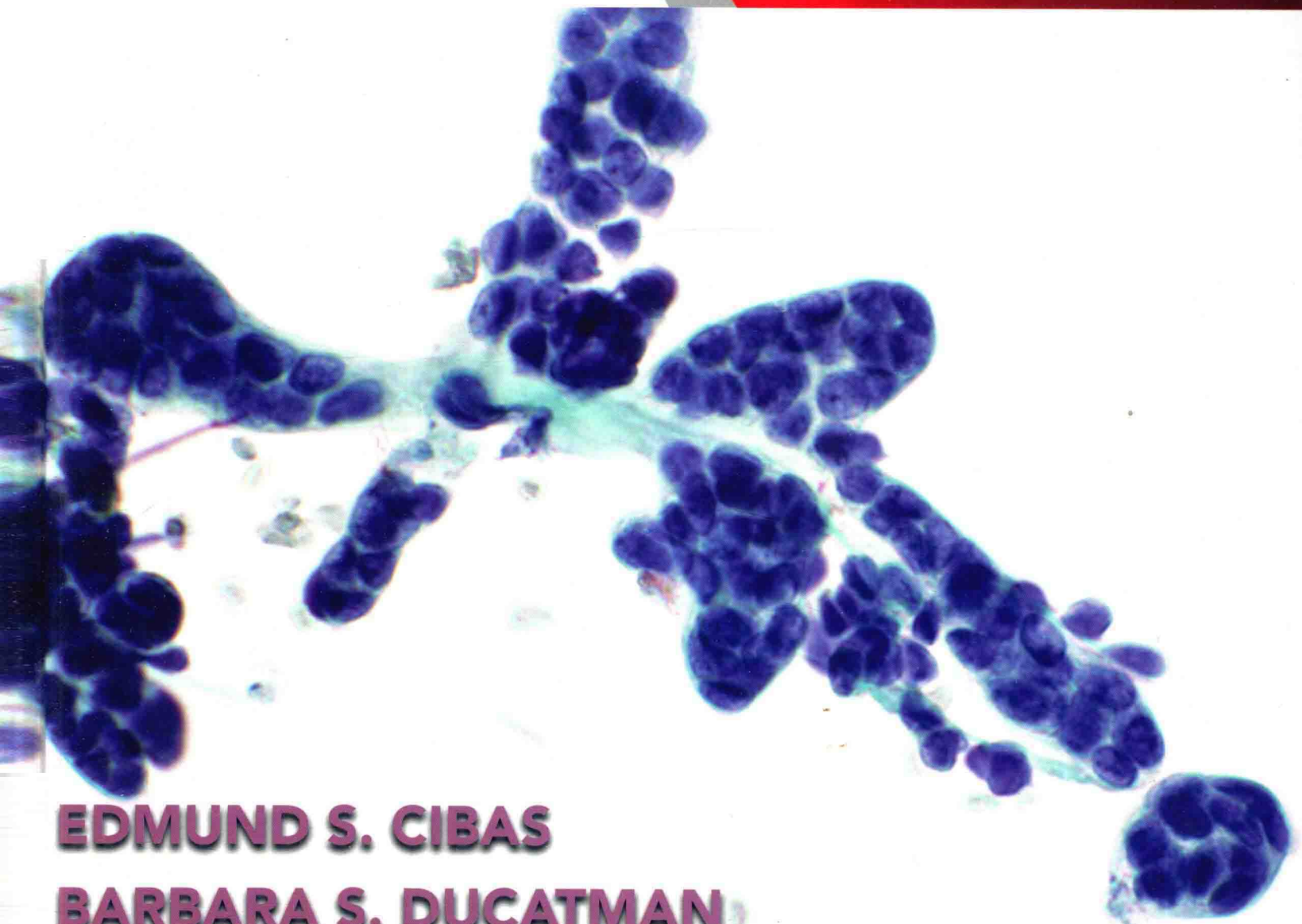

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CYTOLOGY

Diagnostic Principles and Clinical Correlates

Fourth Edition

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Diagnostic Principles and Clinical Correlates

CYTOLOGY

Diagnostic Principles and Clinical Correlates

FOURTH EDITION

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PREFACE

We hope this book will serve as a useful guide for the pathologist in practice and for the trainee—resident or fellow—who is looking to obtain expertise in the subspecialty of cytopathology.

It has been four years since the publication of the third edition of *Cytology: Diagnostic Principles and Clinical Correlates*. Since then, cytology has continued to grow and evolve as a discipline devoted to the diagnosis of cellular tissue obtained by minimally invasive methods (e.g., scraping, brushing, aspiration), thus the need for this updated edition. However, we have retained many of the qualities of the prior editions. This edition again aims to be concise yet comprehensive. We have emphasized brevity and clarity. The text is grounded in an understanding of surgical pathology and current diagnostic terminology. Where relevant, we have illustrated the value of established ancillary studies. Although the book is multi-authored, the chapters follow a similar format: indications, sample collection and preparation methods, recommended terminology for reporting results, accuracy (including common pitfalls that lead to false-negative and false-positive diagnoses), a description of normal elements, and, finally, a how-to guide for the diagnosis of benign and malignant lesions with an emphasis on differential diagnosis. We have retained the bulleted "capsule summaries," particularly for summarizing cytomorphologic features and differential diagnoses. We have continued to emphasize clinical correlation (hence the title). For example, Chapter 1 includes the recently revised guidelines of the American Society for Colposcopy and Cervical Pathology for managing women with abnormal cervical cytologic diagnoses. Good cytologists are those who understand the clinical implications of their interpretations.

A major enhancement of this new edition is the inclusion of a dedicated chapter on fine-needle aspiration technique and specimen handling, accompanied by a video demonstration. We hope trainees and even practicing pathologists will find this especially useful.

Once again, we hope we have conveyed the beauty, strength, and challenge of cytology. With this book we have strived to take some of the mystery out of cytology, but mysteries remain, their solutions still obscure. If this text inspires the reader to explore and even solve some of them, we will consider ourselves doubly rewarded.

Edmund S. Cibas, MD
Barbara S. Ducatman, MD
2013

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We express our deep appreciation to Mr. Dennis Padget of DLPadget Enterprises, Inc., for his help with the complexities of billing in Chapter 18. We relied extensively on his *Pathology Service Coding Handbook* for the information set forth in that chapter. Readers who want more information on pathology coding questions can contact Mr. Padget at DennisPadget@Embarq Mail.com (502-693-5462) for information about subscribing to that comprehensive electronic text.

We are indebted to many members of the staff of the Brigham and Women's Hospital and West Virginia University School of Medicine and Hospital—the cytotechnologists, cytopathologists, and trainees—who inspire us with their devotion to cytopathology and who continue to challenge us. In particular, we acknowledge Dorothy Nappi, CT (ASCP), and Grace Goffi, CT, MIAC, who have helped us train so many pathology residents and fellows over the years. Without their help we would not have our extraordinary collections of cytology teaching cases from which so many of the images in this book are derived.

Finally, to our friends, families, and loved ones, especially Todd Stewart and Alan Ducatman, who tolerated the long evening and weekend hours that deprived them (temporarily!) of a large share of our time. This book would not exist without their love and strength.

Edmund S. Cibas

Barbara S. Ducatman

CONTENTS

chapter 1

CERVICAL AND VAGINAL CYTOLOGY

Edmund S. Cibas

The 20th century witnessed a remarkable decline in the mortality from cervical cancer in many developed countries. This achievement is attributable to the implementation of the Papanicolaou (Pap) test. In the 1930s, before Pap test screening was introduced, cervical cancer was the most common cause of cancer deaths in women in the United States.[1] Today, it is not even in the top 10.[2]

There are approximately 12,000 new cases of cervical cancer in the United States each year, with 4000 deaths.[2] Worldwide, however, the cervical cancer incidence (over 500,000 cases annually) and mortality (275,000 deaths per year) are second only to those for breast cancer.[3] Screening programs, unfortunately, are rudimentary or nonexistent in many parts of the world. Less than 5% of women in developing countries have ever had a Pap test.[4] By contrast, 89% of women in the United States report having had a Pap test in the preceding 3 years.

Around the world, Pap test screening is implemented in two different ways, commonly referred to as

opportunistic versus *organized*.[5] An *organized screening program* is planned at the national or regional level. It specifies a target population and screening intervals and has a mechanism for inviting women to attend screening services, informing them of their result, and referring them for treatment. *Opportunistic screening*, the system in place in the United States, for example, is done independently of an organized or population-based program, on women who are often visiting health services for other reasons. Screening is recommended during a consultation or requested by the woman. Opportunistic screening tends to reach younger, lower-risk women who are attending family planning and antenatal services. It is generally accepted that organized screening is more cost-effective than opportunistic screening, making better use of available resources and ensuring that the greatest number of women benefit.

History of the Papanicolaou Test and Its Current Practice

The Pap test is considered by many to be the most cost-effective cancer reduction program ever devised.[1] Credit for its conception and development goes to George N. Papanicolaou, an anatomist and Greek immigrant to the United States. In 1928 he reported that malignant cells from the cervix can be identified in vaginal smears.[6] Later, in collaboration with the gynecologist Herbert Traut, who provided him with a large number of clinical samples, Papanicolaou published detailed descriptions of preinvasive cervical lesions.[7,8] Pathologists and clinicians initially greeted this technique with skepticism, but by the late 1940s Papanicolaou's observations had been confirmed by others. The Canadian gynecologist J. Ernest Ayre suggested taking samples directly from the cervix with a wooden spatula, rather than from the vagina with a pipette as originally described by Papanicolaou.[9] Eventually, cytologic smears were embraced as an ideal screening test for preinvasive lesions, which, if treated, would be prevented from developing into invasive cancer.

The first cervical cancer screening clinics were established in the 1940s.[10] The Pap test was never evaluated in a controlled, prospective study, but several pieces of evidence link it to the prevention of cervical cancer. First, the mortality rate from cervical cancer fell dramatically after screening was introduced, by 72% in British Columbia[11] and 70% in Kentucky.[12] Second, there was a direct correlation between the intensity of screening and the decrease in mortality. Among Nordic countries, the death rate fell by 80% in Iceland, where screening was greatest; in Norway, where screening was lowest, the death rate fell by only 10%.[13] A similar correlation was observed in high- and low-screening regions of Scotland[14] and Canada.[15] In the United States, the decrease in deaths from cervical cancer was proportional to the screening rates in various states.[16] Finally, women in whom invasive cancer does not develop are more likely to have had a Pap test than women with cancer. In a Canadian study, the relative risk for women who had not had a Pap test for 5 years was 2.7,[17] and screening history was a highly significant risk factor independent of other factors such as age, income, education, sexual history, and smoking. In Denmark, a woman's risk of developing cervical cancer decreased in proportion to the number of negative smears she had had—by 48% with just one negative smear, 69% with two to four negative smears, and 100% with five or more smears.[18]

Screening guidelines differ around the world. In the United States, revised cervical cancer screening recommendations were issued in 2012 by the American College of Obstetricians and Gynecologists (ACOG),[19] the U.S. Preventive Services Task Force (USPSTF),[20] and a consortium of the American Cancer Society, the American Society for Colposcopy and Cervical Pathology, and the American Society for Clinical Pathology (ACS/ASCCP/ASCP).[21] Their guidelines differ in minor ways, but there is general agreement on the larger points, including longer screening intervals and a later age to start screening (age 21) than had been recommended in the past (Table 1.1). The U.S. Department of Health and Human Services (DHHS) offers a web-based National Guideline Clearinghouse that synthesizes the guidelines of the different organizations.[22] The guidelines address women with an average risk for cervical cancer. Women at higher risk—those with a history of cervical cancer, in utero diethylstilbestrol (DES) exposure, and/or immunocompromise (due to organ transplantation, chemotherapy, chronic corticosteroid treatment, or infection with the human immunodeficiency virus [HIV])—may benefit from more frequent screening. Because women with HIV infection/acquired immune deficiency syndrome (AIDS) have higher rates of cervical cancer than the general population, it is recommended that HIV-seropositive women have a Pap test twice during the first year after diagnosis of HIV infection and, if the results are normal,

TABLE 1.1 CERVICAL CANCER SCREENING GUIDELINES IN THE UNITED STATES (FOR WOMEN AT AVERAGE RISK)

Circumstance	Recommendation
Age to begin screening	Age 21. Women younger than age 21 should not be screened, regardless of the age of sexual initiation
Women aged 21 to 29 years	Every 3 years with cytology (liquid-based or conventional) alone
Women aged 30 to 65 years	Every 3 years with cytology alone, or Every 5 years if cotesting with cytology and human papillomavirus (HPV) assay (preferred by ACOG and ACS/ASCCP/ASCP)
Discontinuation of screening	Age 65 years if adequate prior screening and no history of cervical intraepithelial neoplasia (CIN) 2 or higher*
Screening after total hysterectomy	Not recommended if no history of CIN 2 or higher

ACOG, American College of Obstetrics and Gynecology; *ACS/ASCCP/ASCP*, American Cancer Society/American Society for Colposcopy and Cervical Pathology/American Society for Clinical Pathology; *CIN 2*, cervical intraepithelial lesion grade 2.

*ACOG and ACS/ASCCP/ASCP define "adequate prior screening" as *three consecutive negative cytology results or two consecutive negative co-test results within the previous 10 years, with the most recent test performed within the past 5 years.* "No history of CIN 2 or higher" is defined by ACS/ASCCP/ASCP as *within the last 20 years.*

annually thereafter.[23] Adherence to screening guidelines is critical for cervical cancer prevention. In Sweden, for example, women who had not had a Pap smear within the recommended screening interval were at higher risk for development of cervical cancer than those who had been screened (odds ratio 2.52).[24]

In 2012, the ASCCP revised its guidelines for the management of women with abnormal cervical cytology, human papillomavirus (HPV), and histopathologic results.[25] These guidelines, mentioned throughout this chapter in the relevant sections, apply only to women whose abnormalities are detected during screening. Management is individualized for women with postcoital or unexplained abnormal vaginal bleeding, pelvic pain, abnormal discharge, or a visible cervical lesion.

Two prophylactic HPV vaccines provide a new opportunity for cervical cancer prevention. Both vaccines consist of empty protein shells called *viruslike particles* that are made up of the major HPV capsid protein L1. They contain no DNA and are not infectious. One of the vaccines, Gardasil (Merck & Co., Inc.), is a quadrivalent vaccine that protects against HPV types 6, 11, 16, and 18. The other is the bivalent vaccine Cervarix (GlaxoSmithKline), which protects against HPV 16 and 18. They have shown extraordinary efficacy in preventing type-specific histologic cervical intraepithelial neoplasia (CIN) grade 2/grade 3 lesions, with no difference in serious adverse effects from placebo.[26] The vaccines are administered in three doses to females prior to the initiation of sexual activity. Screening guidelines, however, are no different for the vaccinated population than for those not vaccinated. Continued Pap screening, even for the vaccinated population, remains important because these vaccines do not protect against 30% of cervical cancers (i.e., those not related to HPV 16 or 18); the duration of protection is unkown; they are not effective in treating prevalent HPV infections; and the cost of the vaccines might limit their use in some populations. The American Cancer Society recommends routine HPV vaccination principally for females aged 11 and 12 years, and also for females aged 13 to 18 to "catch up" those who missed the opportunity to be vaccinated.[27] According to the 2011 National Immunization Survey of Teens, 53% of female adolescents aged 13 to 17 years in the United States had initiated HPV vaccination, and 35% had completed the recommended three doses.[28]

Sampling and Preparation Methods

To obtain an ideal Pap specimen, the American Cancer Society recommends the following patient instructions[29]:

Patient instructions

- Try not to schedule an appointment for a time during your menstrual period. The best time is at least 5 days after your menstrual period stops.
- Do not use tampons, birth-control foams, jellies, other vaginal creams, or douches for 2 to 3 days before the test.
- Do not have sexual intercourse for 2 days before the test.

Once the patient is positioned, a bivalve speculum of appropriate size is gently inserted into the vagina.[30]

Specimen collection

- The speculum can be lubricated with warm water or sparingly applied water-soluble lubricant.
- Excess mucus or other discharge should be removed gently with a cotton swab.
- The sample should be obtained before the application of acetic acid or Lugol's iodine.
- An optimal sample includes cells from the ectocervix and endocervix.

Water-soluble gel lubricant, if used, should be applied sparingly to the posterior blade of the speculum, avoiding the tip; excessive lubricant can result in an unsatisfactory specimen.[30-34] When visible, different lubricants have different effects and different appearances on cytologic preparations.[34-36] It can be helpful to check any guidelines issued by the manufacturers of liquid-based cytology instruments with regard to recommended lubricants.

There are no clinically important differences between conventional smears and liquid-based cytology (LBC) methods, so either is considered acceptable for cytologic screening.[20,21]

Conventional Smears

Conventional smears are often obtained using the combination of a spatula and brush. The spatula is used first. Although a wooden or plastic spatula is acceptable, the plastic spatula is recommended, because wooden fibers trap diagnostic material.[30] The spatula is rotated at least 360°. The sample can be smeared on one half of a slide and spray fixed (the other half should be covered to avoid coating it with fixative before the endocervical sample is applied). Alternatively, one may set aside the spatula sample momentarily while the endocervical brush sample is obtained.

After the brush is inserted in the endocervical canal, some bristles should still be visible. If it is inserted too far, there may be inadvertent sampling of the lower uterine segment (LUS), which causes diagnostic difficulties because its epithelium resembles a high-grade intraepithelial lesion (HSIL) and adenocarcinoma in situ (AIS). The brush should be rotated gently only one-quarter turn. A larger rotation is unnecessary because the circumferential bristles are in contact with the entire surface the moment the brush is inserted.

The spatula sample, if not already applied and fixed, should be applied to the slide, then the brush sample rolled over the slide, followed by immediate fixation. The two samples can be placed in quick succession on two separate halves of the slide, or the endocervical sample can be rolled directly over the spatula sample, both covering the entire slide. Immediate

fixation (within seconds) is critical in order to prevent air-drying artifact, which distorts the cells and hinders interpretation.

The broomlike brush ("broom") has a flat array of plastic strips contoured to conform to the cervix, with longer strips in the middle. This design allows simultaneous sampling of the endocervix and ectocervix. The long middle strips are inserted into the os until the shorter outer strips bend against the ectocervix. The broom is rotated three to five times. To transfer the material, each side of the broom is stroked once across the slide in a painting motion.

The cotton swab moistened with saline is no longer recommended because its fibers trap cells, reducing the efficiency of cell transfer onto slides.

There are two options for smear fixation. Coating fixatives contain alcohol and polyethylene glycol and are applied by pump sprays, by droppers from dropper bottles, or by pouring from an individual envelope included as part of a slide-preparation kit. Alternatively, the smear can be immersed directly into a container filled with 95% ethanol.

Samples for LBC are obtained as just described, except that instead of smearing the cells on a slide, the collection device is rinsed in a vial containing a liquid fixative. In the United States, the liquid-based Pap test is more common than the smear.

Liquid-Based Cytology

In 1996, the U.S. Food and Drug Administration (FDA) approved the **ThinPrep** (Hologic, Marlborough, MA) as an alternative to the conventional cervicovaginal smear. This was followed 3 years later by approval of the AutoCyte Prep (now **SurePath**) (BD TriPath, Burlington, NC). LBC was an important step in the development of automated Pap screening devices—an improved preparation was needed to minimize cell overlap so that automated instruments would perform better in identifying abnormal cells. But LBC performed so well in clinical trials against conventional smears that it found a market independent of automated screening. Although a number of studies showed an increased detection of cytologic low-grade squamous cell intraepithelial lesion (LSIL) and/or HSIL with LBC,[37] subsequent meta-analyses and prospective randomized trials failed to demonstrate a significant difference between conventional smears and LBC in the detection of histologic CIN 2/3.[38,39] Nevertheless, LBC offers several advantages over conventional smears: the opportunity to prepare duplicate slides and even cell block preparations from the residual sample[40,41]; the option of "out-of-vial" aliquoting for HPV, *chlamydia*, and gonorrhea testing; an improved substrate for automated screening devices; and a thinner cell preparation that most pathologists and cytotechnologists find less tiring to review than smears.

ThinPrep Papanicolaou Test

The practitioner obtains the ThinPrep Pap sample with either a broom-type device or a plastic spatula/endocervical brush combination. The sampling device is swirled/rinsed in a methanol-based preservative solution (PreservCyt) for transport to the cytology laboratory and then discarded. Red blood cells are lysed by the solution. The vials are placed one at a time on the ThinPrep 2000 instrument. The entire procedure (Fig. 1.1*A*) takes about 70 seconds per slide and results in a thin deposit of cells in a circle 20 mm in diameter (contrast with cytospin: diameter = 6 mm). A batch-processing version (the ThinPrep 3000) is also available. It uses the same consumables (filters and solutions) but allows automated processing of 80 samples at one time. In most cases, only a fraction of the sample is used to prepare the slide used for diagnosis. If needed, the residual sample is available for additional ThinPrep slide preparation, cell block preparation, or molecular diagnostic testing (e.g., high-risk HPV, *chlamydia*, gonorrhea).

A multicenter, split-sample study found that the ThinPrep detected 18% more cytologic cases of LSIL and more serious lesions as compared with conventional smears, with no significant difference in the detection of organisms.[42] A number of studies have shown significant increases in the detection of cytologic HSIL after the implementation of the ThinPrep.[37,43-47] Subsequent meta-analyses and a prospective randomized trial, however, failed to demonstrate a significant difference between conventional smears and ThinPrep in the detection of histologic CIN 2/3.[38,39] Data suggest that the ThinPrep is equivalent to the conventional smear in the detection of endocervical AIS and endometrial pathology.[48,49]

The ThinPrep collection vial has been approved by the FDA for testing for HPV, useful for primary screening alongside the Pap (so-called cotesting), and for managing women whose Pap specimen shows atypical squamous cells (ASCs).[25,50]

SurePath Papanicolaou Test

TriPath Imaging (acquired by Becton Dickinson in 2006) developed the SurePath Pap test (formerly AutoCyte Prep) for samples collected in an ethanol-based transport medium. The process is shown in Figure 1.1*B*. In contrast with the ThinPrep method, the practitioner snips off the tip of the collection device and includes it in the sample vial. The equipment to prepare slides includes a Hettich centrifuge and the PrepStain robotic sample processer with computer and monitor. The PrepMate is an optional accessory that automates mixing the sample and dispensing it onto the density reagent. Red blood cells and some leukocytes are eliminated by density centrifugation. In addition to preparing an evenly distributed deposit of cells in a circle 13 mm in diameter, the method incorporates a final staining step that discretely stains each individual slide.

A multicenter, split-sample clinical trial showed a 7.2% increase in the detection of cytologic LSIL and more serious lesions, as well as a significant decrease in the percentage of unsatisfactory specimens.[51] Subsequent meta-analyses, however, failed to demonstrate a significant difference between conventional smears and SurePath in the detection of histologic CIN 2/3.[39]

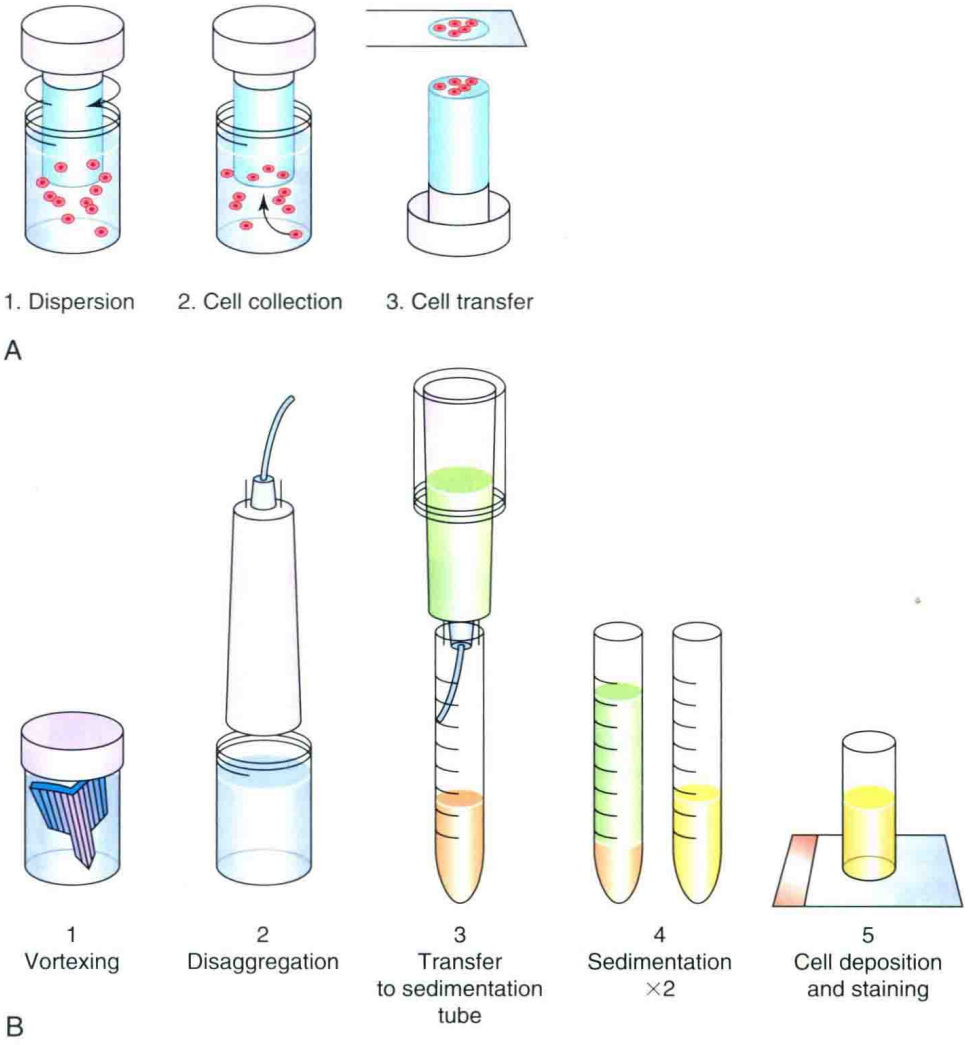

Figure 1.1 Liquid-based slide preparation methods. *A,* ThinPrep method. 1. The sample vial sits on a stage, and a hollow plastic cylinder with a 20 mm diameter polycarbonate filter bonded to its lower surface is inserted into the vial. A rotor spins the cylinder for a few seconds, dispersing the cells. 2. A vacuum is applied to the cylinder, trapping cells on the filter. The instrument monitors cell density on the filter. 3. With continued application of vacuum, the cylinder (with cells attached to the filter) is inverted 180°, and the filter pressed against a glass slide. The slide is immediately dropped into an alcohol bath. *B,* SurePath method. 1. The sample is vortexed. 2. Cell clusters are disaggregated by syringing the sample through a small orifice. 3. The sample is poured into a centrifuge tube filled with a density gradient reagent. 4. Sedimentation is performed in a centrifuge. A pellet is obtained and resuspended, and the sedimentation is repeated. 5. The tubes are transferred to the PrepStain instrument, where a robotic arm transfers the fluid into a cylinder. Cells settle by gravity onto a cationic polyelectrolyte-coated slide. The same robotic arm also dispenses sequential stains to individual cylinders.

Automated Screening

Historical Overview

Automated cytology screening devices have been under development since the 1950s. The first computerized screening system was developed in the United States by Airborne Instruments Inc. and was called the Cytoanalyzer.[52] In preclinical trials it did not perform as well as expected, and the project was discontinued. The difficulty of the task was soon appreciated, especially the inherent problems with analyzing smears prepared in the conventional manner. Despite setbacks, research into cervical cytology screening continued throughout the following decades, with the development of the TI-CAS,[53] Quantimet,[54] BIOPEPR,[55] CERVIFIP,[56] CYBEST,[57] DIASCANNER,[58,59] FAZYTAN,[60] and LEYTAS.[61] Some of these instruments are now in museums, but others have served as prototypes for systems that are now commercially available.

In the 1990s, researchers in the United States and Canada established private enterprises supported by venture capital in order to develop a commercial automated screening instrument. Foremost in the field were AutoCyte (formerly Roche Image Analysis Systems), Cytyc, Neopath, and Neuromedical Systems. A three-way merger took place in 1999, when AutoCyte, after purchasing the intellectual property of Neuromedical Systems, merged with Neopath to form a new company called TriPath Imaging, acquired in 2006 by Becton Dickinson. In 2007, Cytyc Corporation, developer of the ThinPrep Pap Test and ThinPrep Imaging System, merged with Hologic Inc. and became a wholly owned subsidiary of Hologic.

In 1998, the FDA approved the AutoPap System (now called the FocalPoint Slide Profiler; BD TriPath Imaging, Burlington, NC) as a primary screener for conventional cervicovaginal smears, followed by approval in 2002 for use with SurePath slides. In 2003, the FDA approved the ThinPrep Imaging System (Hologic, Marlborough, MA) as a primary screener for ThinPrep Pap slides, and in 2008 it approved the FocalPoint Guided Screening (GS) Imaging System. Neither is approved in the United States for automated screening of nongynecologic cytology specimens.

ThinPrep Imaging System

The ThinPrep Imaging System (TIS) uses the principle of *location-guided screening* to aid the cytotechnologist in reviewing a ThinPrep Pap slide. TIS consists of two

Figure 1.2 Automated cytology screening devices. *A,* ThinPrep Imaging System: the imager. The imager consists of *(left to right)*: the imaging station, an image processor and server, and a user interface consisting of a monitor, keyboard, and mouse. *B,* ThinPrep Imaging System: the Review Scope. Imaging data are electronically linked to a customized microscope called the Review Scope. After the ThinPrep slides have been imaged, they are brought to the RS for location-guided review. In addition to a microscope, there is a console (with display and keypad) and a navigator pod. *C,* BD FocalPoint Slide Profiler. The FocalPoint Slide Profiler consists of two main components *(left to right)*: the workstation (computer, monitor, keyboard, mouse, modem, and printer) and the floor-standing instrument (slide processor). *D,* BD FocalPoint Guided Screening Review Station. After SurePath slides have been imaged, they are brought to the Review Station for location-guided review. Imaging data are electronically linked to a customized microscope. In addition to the microscope, there is a barcode scanner and a monitor with keyboard and mouse. (*A* and *B* courtesy Hologic, Inc. and affiliates. *C* and *D* courtesy BD Diagnostics Inc.)

components, the image processor ("imager") and the Review Scope (Fig. 1.2*A* and *B*). Stained and coverslipped ThinPrep slides are placed in a cartridge (each cartridge holding 25 slides), and up to 10 cartridges are loaded onto the bench-top imager. The imager has the capacity to screen more than 300 slides per day. It scans the slides and identifies 22 fields of view (FOV) on each slide that, based on optical density measurements and other features, are the most likely to harbor abnormal cells. The *x* and *y* coordinates of the 22 FOV are stored in a database and retrieved at a later time. The server is electronically linked to one or more Review Scopes in the laboratory. A Review Scope resembles a standard microscope but is augmented with an automated stage, a pod that controls the stage and objectives, and a keypad. The scope also has a camera that reads the slide identifier when the slide is loaded onto the stage. When a valid slide identifier is recognized, the server sends its coordinate information to the scope, permitting the cytotechnologist to navigate to the 22 FOV using the pod. Navigation to each FOV is done geographically—that is, using the shortest distance from one FOV to the next. The cytotechnologist uses the pod to advance forward or return back through the FOV, changing objectives as needed. If no abnormal cells are found in any of the FOV, the case has been completed and can be reported as negative. If any abnormal cells are found in any of the FOV, a review of the entire slide must be performed. This can be done using the autoscan function on the Review Scope, with preset, customized user screening preferences. The Review Scope has both electronic and physical slide dotting capabilities.

The accuracy of the TIS was evaluated in a clinical trial at four laboratories. ThinPrep slides were first screened manually, and the results recorded. They were

then rescreened using the TIS. Truth adjudication was performed by expert review of all abnormal cases and a proportion of negative slides. The TIS detected significantly more abnormal slides (atypical squamous cells of undetermined significance [ASC-US] or greater) than manual review (82% versus 76%).[62] A later split-sample study comparing conventional smear cytology versus the TIS for ThinPrep slides showed a significantly higher detection rate of histologic HSIL (CIN 2/3) with the TIS.[63]

Because 22 FOV represent approximately 25% of the ThinPrep cell spot,[64] implementation of the TIS enhances productivity.[62,65,66]

Implementing the TIS requires adopting the proprietary ThinPrep Pap stain, to which some adjustment is necessary because it yields darker nuclear staining of metaplastic and endocervical cell clusters than most traditional Pap stains. The TIS does not eliminate false-negatives, which are still encountered, albeit less frequently than in the absence of imaging.[62] A number of postapproval studies have shown significant increases in the detection of cytologic LSIL and HSIL after implementation of the TIS.[67-69]

BD FocalPoint Guided Screening Imaging System

The BD FocalPoint Guided Screening (GS) Imaging System (Fig. 1.2C and *D*) uses programmed algorithms to measure cellular features like nuclear size, integrated optical density, nuclear-to-cytoplasmic ratio, and nuclear contour—morphologic features established using planimetry and ocular micrometry for the diagnosis of squamous and glandular lesions.[70]

AutoPap, the predecessor of the BD FocalPoint GS Imaging System, was originally intended as a primary screening device that would eliminate the need to manually screen as many as one half of all smears. It was temporarily redesigned as a quality control rescreening device called the AutoPap 300 QC System and obtained FDA approval for this function in 1995. The AutoPap 300 QC System did not find a wide audience, however, and became obsolete in the year 2000. A redesign resulted in a new instrument (the AutoPap System-Primary Screener, later renamed BD FocalPoint Slide Profiler) which obtained FDA approval as a primary screening device in 1998. In this mode, the device is used in the initial screening of smears. It identifies up to 25% of slides as requiring "no further review." Of the remaining slides that require manual review, it also identifies at least 15% for a second manual review, which may be used as a substitute for the 10% review of negative Paps required of all U.S. laboratories (see Chapter 18). A barcode is applied to each slide, and slides are loaded into slide trays. Up to 288 slides can be loaded at a time (8 slides per tray, 36 trays). Each slide is analyzed using preset algorithms at ×4 magnification for a visual map of the entire slide, then 1000 fields are captured at ×20 magnification. After analysis, the device assigns a score (from 0 to 1.0) to each slide according to the likelihood of an abnormality. Slides with scores below a cut off are considered "no further review," and those above the cut-off are triaged for full manual review. Any slide deemed unsuitable for analysis because of preparation or coverslipping problems requires manual review.

The accuracy of the BD FocalPoint Slide Profiler was evaluated in a clinical trial at five laboratories.[71] Each slide was first evaluated in the conventional manner. The same slides were then processed by the AutoPap System, which detected significantly more abnormal slides (ASC-US or greater) than conventional practice (86% versus 79%). Of importance, the BD FocalPoint Slide Profiler is not approved for women at high risk for cervical cancer. Thus, a laboratory that uses the BD FocalPoint Slide Profiler for primary screening must set aside all Paps from high-risk women for manual screening. It is up to the laboratory to define what constitutes a Pap from a high-risk patient. False-negative results are occasionally encountered with the BD FocalPoint Slide Profiler. In the clinical trial, there were 10 false-negatives (5 ASC-US, 4 LSILs, and 1 HSIL) in the 1182 cases considered "no further review," and another study found 9 false-negatives (5 ASC-US and 4 LSILs) in the 296 cases considered "no further review."[72] The productivity gain is modest, because in practice the FocalPoint Slide Profiler archives only about 16% to 17% of Paps without full manual review.[71,73]

The most recent phase in BD FocalPoint development occurred in 2008 with FDA approval of the BD FocalPoint GS Imaging System. The BD FocalPoint GS Imaging System consists of the BD FocalPoint Slide Profiler plus a BD FocalPoint GS Review Station and, like the TIS, uses the principle of location-guided screening to aid the cytotechnologist in reviewing a slide. A SurePath slide is first examined by the BD FocalPoint Slide Profiler, which uses algorithms to identify the 10 FOV most likely to harbor abnormal cells. These FOV slides are presented to a cytotechnologist for review at the microscopic Review Station; if no abnormality is detected in the FOV, the slide is reported as negative without any further review. But if any abnormality is seen in any of the FOV samples, or if specimen adequacy cannot be confirmed, the slide is triaged for full manual review.

The accuracy of the BD FocalPoint GS Imaging System was evaluated in a clinical trial at four laboratories. The detection of cytologic HSIL+ increased by 19.6% and of cytologic LSIL+ by 9.8% in the computer-assisted arm, with small but statistically significant decreases in specificity. For cytologic ASC-US+ sensitivity and specificity, the study arms were not statistically different.[74] As with the TIS, implementation of the BD FocalPoint GS Imaging System enhances productivity.[75]

Accuracy and Reproducibility

The sensitivity of cytology for detecting preinvasive squamous and glandular lesions is difficult to establish, but it is clearly far from perfect. Most studies of preinvasive lesions suffer from verification bias (i.e., cases are referred for biopsy on the basis of an abnormal smear, and biopsy is not performed in women with negative Pap test results). The few relatively unbiased

studies show that the mean sensitivity of the Pap test is 47% (range 30% to 80%), and the mean specificity is 95% (range 86% to 100%).[76]

The sensitivity of cytology is less than ideal for invasive cancers as well, and estimates range widely (16% to 82%). Many women with cervical cancer have a history of one or more negative smears.[77-88] The relative contributions of sampling and laboratory error vary from one study to another and likely depend on how carefully retrospective rescreening is performed.

False-positive diagnoses of cervical cancer occur in 10% to 15% of cases.[89,90] The chief culprits are the atrophic smear with benign squamous atypia in a granular, pseudonecrotic background; reparative changes; and keratinizing HSILs.

The interobserver reproducibility of cytologic interpretations is also less than perfect. In a large study of women, most of whom had mild cytologic abnormalities, the unweighted κ statistic for four categories of diagnosis—negative, atypical, LSIL, and HSIL—was 0.46, indicating moderate reproducibility.[91] (Roughly, a κ of 0 or less represents poor agreement; 0 to 0.2, slight agreement; 0.2 to 0.4, fair agreement; 0.4 to 0.6, moderate agreement; 0.6 to 0.8, very good agreement; and 0.8 to 1.0, almost perfect agreement.) In the same study, the reproducibility of histologic interpretations of cervical biopsies, also for four categories of diagnosis, was identical (0.46). The greatest disagreement with Paps involved those originally interpreted as showing ASC-US; the second reviewer agreed with only 43% of cases. The greatest disagreement with biopsies involved those originally interpreted as CIN 1; the second reviewer concurred in only 43% of cases.[91]

A graphic demonstration of the relative reproducibility of various cytologic findings is available on the Bethesda System Web Atlas, which contains the results of the Bethesda Interobserver Reproducibility Project. A large number of images were reviewed by hundreds of observers, who were asked to place the images into one of the Bethesda System categories. The results are displayed for each image as a histogram.[92]

Diagnostic Terminology and Reporting Systems

Papanicolaou devised a numerical system for reporting cervical smears, which was originally intended to convey his degree of suspicion that the patient had cancer: **class I,** absence of atypical or abnormal cells; **class II,** atypical but no evidence of malignancy; **class III,** suggestive of but not conclusive for malignancy; **class IV,** strongly suggestive of malignancy; and **class V,** conclusive for malignancy. Over time, however, the Papanicolaou class system underwent many modifications and was not used in a uniform fashion.[93] It nevertheless persisted in many laboratories well into the 1980s. In other laboratories it was replaced (or supplemented) by descriptive terms borrowed from histologic classifications of squamous lesions. Squamous cancer precursors were originally divided into **carcinoma in situ**, a high-risk lesion of immature, undifferentiated atypical cells, and **dysplasia** (subdivided into mild, moderate, and severe), the latter a lower-risk lesion of more mature squamous cells. In the 1960s, Richart challenged the duality of dysplasia/carcinoma in situ and proposed a new term, **cervical intraepithelial neoplasia** (CIN). CIN was graded from 1 to 3, but Richart believed that CIN 1 (mild dysplasia) had a strong propensity to progress to CIN 3 and cancer. The high rate of progression found in his study most likely related to stringent entry criteria: for inclusion, CIN 1 had to be confirmed on three consecutive Paps.[94] The study data showed a higher progression rate for mild dysplasia than most other natural history studies.[95] The CIN concept was highly influential, however, and for many years squamous precursors were treated as much on the basis of their size and location as on their grade.

In 1989, the Bethesda System was introduced to standardize the reporting of cervical cytology results and incorporate new insights gained from the discovery of HPV.[96] The name for a squamous cancer precursor was changed to **squamous intraepithelial lesion (SIL)**, subdivided into only two grades (low and high), based on the evolving understanding of the biology of HPV. In this system, LSIL encompasses CIN 1, and HSIL encompasses CIN grades 2 and 3. This was a shift away from the CIN concept, one based on a reevaluation of the existing evidence, which demonstrated that most LSILs are, in fact, transient HPV infections that carry little risk for oncogenesis, whereas most HSILs are associated with viral persistence and a significant potential for progression to invasive cancer.

The first Bethesda System workshop, in 1988, was followed by two others, in 1991 and 2001, which made modifications to the original framework and terminology. The 2001 workshop broadened participation by using a dedicated website on the Internet, and an electronic bulletin board received more than 1000 comments regarding draft recommendations. The 2001 Bethesda System, like its predecessors, recommends a specific format for the cytology report, starting with an explicit statement on the adequacy of the specimen, followed by a general categorization and an interpretation/result.[97,98]

The Bethesda System

Specimen Adequacy

One of the most important advances of the Bethesda System is its recommendation that each Pap report begin with a statement of adequacy. In 1988, the Bethesda System proposed three categories for specimen adequacy: "satisfactory," "less than optimal" (renamed "satisfactory but limited by ...." in 1991), and "unsatisfactory." The 2001 Bethesda System eliminated the middle category because it was confusing to clinicians and prompted unnecessary repeat Pap tests. Nevertheless, the 2001 Bethesda System advocates mentioning the presence or absence of a transformation zone component and permits comments on obscuring elements. The 2001 Bethesda System criteria for adequacy are listed in Table 1.2. They are somewhat arbitrary, because scientific data on adequacy are