The Bethesda System for Reporting Cervical Cytology

Definitions, Criteria, and Explanatory Notes

Third Edition

Ritu Nayar
David C. Wilbur
Editors

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Foreword

It is a privilege, a pleasure, and something of a surprise for me to write this Foreword to the third edition of the Cervical Cytology Bethesda System Atlas. I never imagined that a small meeting on the campus of the National Institutes of Health in Bethesda, Maryland, one snowy weekend in December 1988 would begin a process that has changed the practice of cervical cytology – in both the laboratory and the clinician's office – around the world. This third edition of the atlas continues that evolution, presenting the latest refinements to the Bethesda System (TBS) in a convenient easy-to-use reference designed to be accessible for cytopathologists and cytotechnologists regardless of laboratory setting.

The initial Bethesda System workshop was convened to address a well-recognized but seemingly intractable problem of variability in laboratory reports of Papanicolaou smears [1]. Different laboratories used a multiplicity of terms including, in many cases, Pap class numbers, with confusing and idiosyncratic modifications, or dysplasia terminology with multiple, poorly reproducible gradations including a biologically inaccurate distinction between changes induced by human papillomavirus (HPV) and what was considered "true dysplasia." Additionally, a non-reproducible distinction between severe dysplasia and carcinoma in situ was sometimes used clinically to decide if a hysterectomy should be performed.

The first Bethesda workshop, ably chaired by Dr. Robert Kurman, convened roughly three dozen laboratorians, clinicians, and research scientists with the goal of finding a better way. Over 2 days, the following fundamental principles emerged that have guided the Bethesda System from that day to this:

1. Terminology used by the laboratory must communicate appropriate and clinically relevant information to the clinician
2. Terminology should be consistent from one laboratory to another and reasonably reproducible in practice but at the same time be flexible enough to be adapted in a wide variety of laboratories and geographic settings
3. Terminology should be continuously updated to reflect the most current understanding of the pathobiology of cervical neoplasia and integrate advances in laboratory practice

With these principles in mind, the workshop participants developed terminology based on the underlying pathobiology of the morphologic changes of cervical

epithelial abnormalities. Squamous intraepithelial lesion (SIL) with only two gradations (low and high grade) reflected the different biologic states of productive HPV infections versus lesions with a higher risk of transitioning to precancer and ultimately cancer. In addition to the SIL terminology, TBS also introduced the concept of a "statement of adequacy" of the specimen as an integral part of the report and an important quality assurance element. The new terminology was named after the location of the workshop in Bethesda, Maryland.

Fast-forward 25 years:

Additional Bethesda System workshops were convened in 1991 and 2001, and the first two editions of this atlas were published in 1994 and 2004 [2, 3]. Each of these events was part of the continuing evolution of both scientific knowledge and clinical practice, in particular:

1. A major recommendation from the 1991 workshop was that criteria should be developed for the diagnostic terms and for the determination of specimen adequacy, which led to the publication of the first atlas [2].
2. The workshop in 2001 was the first to utilize the Internet in order to provide everyone an opportunity for input; over 2,000 comments were considered prior to the meeting, which then brought together over 400 participants including representatives from over two dozen countries [4].
3. Developments in laboratory practice and the transition for many to liquid-based cytology led to incorporating images and criteria specific to these preparations in the 2004 atlas [3].

Of all the changes introduced by TBS, none has been as controversial as "atypical squamous cells of undetermined significance" or ASC-US. ASC-US highlighted the inherent limitations of morphologic interpretation. Cytologic findings may be equivocal, resulting in frustration for clinicians who need to be able to make clear-cut management decisions. As ASC-US was (and still is) the most common cytologic abnormality reported for millions of women in the USA annually, this posed a significant clinical problem and threatened to overwhelm the available colposcopy services.

In response, the US National Cancer Institute sponsored a clinical trial, the ASCUS-LSIL Triage Study, or ALTS, to resolve the question of best practice [5]. The results of ALTS established molecular testing for HPV as the most cost-effective approach to clarify equivocal cytologic findings. HPV testing is now firmly integrated into algorithms both for primary cervical screening and cytology triage.

The results of ALTS and other clinical research have, in turn, informed the development of clinical management algorithms involving dozens of organizations and professional societies, spearheaded by the American Society for Colposcopy and Cervical Pathology, most recently in 2012 [6]. At a time when there were few test options for screening and evaluation of abnormal findings, management algorithms consisted of linear branch points based on a sequence of test results. With the multiplicity of testing options currently available, as well as additional assays on the horizon, various combinations of cytologic, molecular, and/or histopathologic test findings must now be integrated in order to determine an individual woman's risk for precancer/cancer and – based on that level of risk – her

appropriate management. A new chapter on a risk assessment-based management has been added to this atlas.

Beyond the field of cervical cytology, standardized terminology systems have now been developed for cytology of other body sites including thyroid [7] and pancreas [8], and most recently urine [9]. The two-tier terminology used in TBS has also been recommended for reporting histopathology of HPV-related squamous lesions of the lower anogenital tract [10, 11].

Terminology must evolve to keep pace with our insights into the basis of disease, to be responsive to the needs of the laboratory and clinician for clear communication, and ultimately to best serve women's health. True to the spirit of the underlying principles that guided the first Bethesda workshop, this third edition of the atlas refines the application of the Bethesda terminology based on experience gathered over the past decade, especially related to the morphology of liquid-based preparations and use of TBS in clinical practice.

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Introduction

In the past decade, since the publication of the second edition of the Bethesda Atlas in 2004, considerable experience has been gained with the use and impact of the Bethesda terminology for cervical cytology in clinical practice. This includes additional experience with morphology on liquid-based preparations, further insights into HPV biology, implementation of HPV vaccination, and updated guidelines for cervical cancer screening and the management of abnormal cervical cytology and cancer precursors. Thus 2014 seemed to be the appropriate time for a review and update of the 2001 Bethesda System terminology and incorporation of revisions and additional information into this third edition of the Bethesda Atlas for cervical cytology.

Despite recent concern about the demise of the Papanicolaou test, as it gradually yields its role as a primary cervical cancer screening test to HPV and other biomarker testing, cervical cytology remains the most successful cancer prevention program ever devised. Its specificity will remain the cornerstone of future screening regimens, including those in women who have received HPV vaccination. Additionally, in many settings, cervical cytology will continue to be the first line screening test based on resources and local preferences. Hence, updating and further refinement of morphologic criteria for the great variety of entities seen in cervical cytology, both neoplastic and non-neoplastic, is an important function of this edition. Wide dissemination of this comprehensive and relatively inexpensive atlas will therefore serve to maximize the overall value of the test in all practice settings.

Since minimal changes were anticipated to the terminology recommended by the 2001 Bethesda System (TBS), there was no consensus workshop held in association with the 2014 Bethesda System update. Therefore, Dr. Ritu Nayar, President of the American Society of Cytopathology (ASC) in 2014, appointed a task force, chaired by Dr. David Wilbur (ASC President in 2002), which was comprised of a relatively small group of cytopathologists and clinicians/epidemiologists in order to expeditiously accomplish this task. Following literature review and formulation of the proposed new and expanded content for the atlas, a widely advertised Internet-based public open comment period was initiated within the international cytopathology community for a 3.5-month period lasting from March through mid-June of 2014. A total of 2454 responses were received from individuals in 59 countries spread over a broad demographic, on proposals from each of the atlas's 12 chapter-based

surveys. Excellent feedback was gathered on the proposed updates, which was compiled and reviewed by the chapter-based task force working groups. This process culminated in refinement of positions and content, which were then incorporated into the 2014 Bethesda System and this accompanying atlas.

This new edition of the atlas expands on the popular features of the prior editions [1, 2]. A portion of the text and images from the first and second editions have been retained for this edition, and credit is attributed to the individuals who participated in the 1988, 1991 and 2001 Bethesda Workshops and those who contributed to the resultant 1994 and 2004 Bethesda atlases (see Acknowledgments section). This edition has 12 chapters, 6 of which correspond to the major Bethesda interpretive categories, with the remainder being dedicated to other malignant neoplasms, anal cytology, reporting of adjunctive testing, computer-assisted screening, educational notes, and a new chapter on cervical cancer risk assessment. Each chapter consists of a background discussion, a description of definitions and cytologic criteria, brief explanatory notes that cover difficult morphologic patterns and mimics of epithelial lesions (where applicable), sample reports, and selected references. Cytologic criteria are described in general for all specimen types in every chapter, followed by any significant differences related to specific preparation types. (*Note that TBS does not endorse any particular methodology or manufacturer(s) for specimen collection, computer-assisted screening, adjunctive HPV or other testing).* New to this edition are increased content on basic disease biology as it pertains to each entity and discussions of the current clinical management guidelines.

Over 1000 images were evaluated for this atlas, including the 186 images from the second edition. The images went through a multistage review process; first by the relevant chapter group, and secondly by a cytopathologist/cytotechnologist subgroup of the Bethesda 2014 Task Force. Dr. Daniel Kurtycz is credited with the management of images collected for this edition of the atlas. The 370 illustrations in this third edition represent a spectrum of morphologic changes seen on both conventional smears and liquid-based preparations (LBPs); 56% are new images and 44% are from the prior two editions; 40% are conventional preparations and 60% are from LBPs. For LBP specimen illustrations, the figure legends specify which of the two commonly used methods is illustrated: ThinPrep™ (Hologic, Marlborough, MA) or BD SurePath™ (BD Diagnostics, Durham NC). Some images represent classic examples of an entity whereas others were selected to illustrate interpretive dilemmas or "borderline" morphologic features that may not be interpreted in the same way by all cytologists. A greater number and variety of "normal" findings as well as mimics of classic epithelial abnormalities are included in the third edition in order to provide a more complete representation of the morphologic variations that can be appreciated in cervical cytology specimens.

Prior to the publication of the second edition [2], selected images were posted on a website open to cytopathologists and cytotechnologists worldwide. This process was designed to evaluate inter-observer variability and to provide an educational tool for cytologists. Results of the Bethesda Interobserver Reproducibility Study (BIRST) can be viewed online and have also been published [3, 4]. To build on the information gathered from our experience with the BIRST project in 2003, we posted 85 of the

images from this atlas as "unknowns" on a website open to the cytopathology community. Data from this effort, in which over 850 participants submitted their answers online prior to the publication of this atlas, provides a realistic gauge of interpretive reproducibility. Information regarding the results of this exercise is available on the ASC website at www.cytopathology.org. While knowledge of normal morphology, its variations and epithelial abnormalities is essential, some degree of interobserver and interlaboratory variability in interpretation will always remain a reality [4, 5].

In parallel with the development of this third edition, a Bethesda 2014 website resource has also been developed by an ASC Bethesda Website Task Force under the direction of Drs. Daniel Kurtycz and Paul Staats. In addition to displaying all the illustrations that are used in this atlas, the website will contain many other examples of presentations and entities that could not be provided in this print version. The website group will also be exploring new avenues for delivery of the content which has been assembled during this update process. For further information on the Bethesda web atlas please go to the educational resources page on the American Society of Cytopathology website [6].

Although the Bethesda System was developed primarily for cervical cytology, specimens from other sites in the lower anogenital tract, such as the vagina and anus, may be reported using similar terminology. As in the 2001 Bethesda System, the terms "interpretation" or "result" are recommended instead of "diagnosis" in the heading of the cervical cytology report. This terminology is preferred because cervical cytology should be viewed primarily as a "*screening test, which in some instances may serve as a medical consultation by providing an interpretation that contributes to a diagnosis*." A patient's final diagnosis and management plan integrate not only the cervical cytology result but also the history, clinical findings, and other laboratory results such as molecular/biomarker testing and biopsy interpretations [2].

As in prior editions, the current editors and authors have committed to making the third edition affordable, and hence, widely accessible to all including practitioners in low resource environments. No honoraria or royalties will be accepted by the editors/authors for this work. The editors, the 2014 Bethesda System Task Force members, and all the dedicated cytologists who have contributed to this wonderful project over the past quarter of a century are delighted to come together to thank Drs. Diane Solomon and Robert Kurman for their pioneering vision in initiating the organization and implementation of the Bethesda System in 1988 [7, 8]. Indeed Bethesda's contributions and impact on the field of cervical cancer go far beyond just standardized reporting terminology. The Bethesda System formed the bedrock for the furthering of our understanding of HPV biology and provided the framework necessary for the development of systematic and evidence-based cervical cancer screening and management guidelines [8]. And finally, Bethesda brought the world together with one cytologic voice – now able to effectively communicate scientific and clinical data where previously such was difficult, if not impossible. Because of Bethesda, the interpretation of a high grade squamous intraepithelial lesion in the United States is based on exactly the same criteria as in India or anywhere else. On behalf of the American Society of Cytopathology, we, as a group are pleased to be

a part of this ongoing process and hope that the 2014 Bethesda System update and this corresponding expanded atlas will prove useful in your practice.

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The 2014 BETHESDA SYSTEM FOR REPORTING CERVICAL CYTOLOGY

SPECIMEN TYPE:
Indicate conventional smear (Pap smear) vs. liquid-based preparation vs. other

SPECIMEN ADEQUACY

- Satisfactory for evaluation (*describe presence or absence of endocervical/transformation zone component and any other quality indicators, e.g., partially obscuring blood, inflammation, etc.*)
- Unsatisfactory for evaluation . . . (*specify reason*)
  - Specimen rejected/not processed (*specify reason*)
  - Specimen processed and examined, but unsatisfactory for evaluation of epithelial abnormality because of (*specify reason*)

GENERAL CATEGORIZATION (optional)

- Negative for Intraepithelial Lesion or Malignancy
- Other: See Interpretation/Result (*e.g., endometrial cells in a woman ≥45 years of age*)
- Epithelial Cell Abnormality: See Interpretation/Result (*specify* 'squamous' *or* 'glandular' *as appropriate*)

INTERPRETATION/RESULT

NEGATIVE FOR INTRAEPITHELIAL LESION OR MALIGNANCY
(W*hen there is no cellular evidence of neoplasia, state this in the General Categorization above and/or in the Interpretation/Result section of the report--whether or not there are organisms or other non-neoplastic findings*)

NON-NEOPLASTIC FINDINGS (*optional to report optional to report; list not inclusive*)

- Non-neoplastic cellular variations
  - Squamous metaplasia
  - Keratotic changes
  - Tubal metaplasia
  - Atrophy
  - Pregnancy-associated changes

- Reactive cellular changes associated with:
  - Inflammation (includes typical repair)
    - Lymphocytic (follicular) cervicitis
  - Radiation
  - Intrauterine contraceptive device (IUD)
- Glandular cells status post hysterectomy

ORGANISMS
- *Trichomonas vaginalis*
- Fungal organisms morphologically consistent with *Candida* spp.
- Shift in flora suggestive of bacterial vaginosis
- Bacteria morphologically consistent with *Actinomyces* spp.
- Cellular changes consistent with herpes simplex virus
- Cellular changes consistent with cytomegalovirus

OTHER

- Endometrial cells (*in a woman ≥45 years of age*)
  (*Specify if "negative for squamous intraepithelial lesion"*)

EPITHELIAL CELL ABNORMALITIES

SQUAMOUS CELL

- Atypical squamous cells
  - of undetermined significance (ASC-US)
  - cannot exclude HSIL (ASC-H)
- Low-grade squamous intraepithelial lesion (LSIL)
  (*encompassing: HPV/mild dysplasia/CIN 1*)
- High-grade squamous intraepithelial lesion (HSIL)
  (*encompassing: moderate and severe dysplasia, CIS; CIN 2 and CIN 3*)
  - with features suspicious for invasion (*if invasion is suspected*)
- Squamous cell carcinoma

GLANDULAR CELL

- Atypical
  - endocervical cells (NOS *or specify in comments*)
  - endometrial cells (NOS *or specify in comments*)
  - glandular cells (NOS *or specify in comments*)
- Atypical
  - endocervical cells, favor neoplastic
  - glandular cells, favor neoplastic

- Endocervical adenocarcinoma in situ
- Adenocarcinoma
  - endocervical
  - endometrial
  - extrauterine
  - not otherwise specified (NOS)

OTHER MALIGNANT NEOPLASMS: *(specify)*

ADJUNCTIVE TESTING

Provide a brief description of the test method(s) and report the result so that it is easily understood by the clinician.

COMPUTER-ASSISTED INTERPRETATION OF CERVICAL CYTOLOGY

If case examined by an automated device, specify device and result.

EDUCATIONAL NOTES AND COMMENTS APPENDED TO CYTOLOGY REPORTS (*optional*)

Suggestions should be concise and consistent with clinical follow-up guidelines published by professional organizations (references to relevant publications may be included).

Acknowledgements

Bethesda System Committee Members and Contributors to Bethesda Atlas, First edition
(Kurman RJ, Solomon D (Eds). The Bethesda System for Reporting Cervical/Vaginal Cytologic Diagnoses. *Definitions, Criteria, and Explanatory Notes for terminology and Specimen Adequacy*. New York: Springer-Verlag, 1994).

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Contents

Abbreviations

ACOG	American College of Obstetricians and Gynecologists
ACS	American Cancer Society
AGC	Atypical glandular cells
AIN	Anal intraepithelial neoplasia
AIS	Adenocarcinoma in situ
ALTS	ASCUS–LSIL Triage Study
APK	Atypical parakeratosis
ASC	Atypical squamous cells
ASCCP	American Society for Colposcopy and Cervical Pathology
ASC-H	Atypical squamous cells cannot exclude an HSIL
ASC-US	Atypical squamous cells of undetermined significance
ASIL	Anal squamous intraepithelial lesions
CAP	College of American Pathologists
CDC	Centers for Disease Control
CIN	Cervical intraepithelial neoplasia
CMV	Cytomegalovirus
cNPV	Complement of the negative predictive value
CP	Conventional preparation
DARE	Digital anorectal exam
DES	Diethylstilbestrol
ECA	Epithelial cell abnormality
EC/TZ	Endocervical/transformation zone
FDA	Food and Drug Administration
FOV	Fields of view
HCG	Hyperchromatic crowded groups
hpf	High–power field
HPV	Human papillomavirus
HRA	High–resolution anoscopy
hrHPV	High–risk human papillomavirus
HSIL	High–grade squamous intraepithelial lesions
IUD	Intrauterine contraceptive device
LAST	Lower Anogenital Squamous Terminology
LBP	Liquid–based preparation
LEEP	Loop electrosurgical excision procedure

LMP	Last menstrual period
LSIL	Low–grade squamous intraepithelial lesion
LUS	Lower uterine segment
MMMT	Malignant Müllerian mixed tumor
N/C	Nuclear/cytoplasmic
NILM	Negative for intraepithelial lesion or malignancy
NOS	Not otherwise specified
nsc	Nucleated squamous cells
PNET	Ewing/primitive neuroectodermal tumors
PPV	Positive predictive value
SCC	Squamous cell carcinoma
SCJ	Squamocolumnar junction
SIL	Squamous intraepithelial lesion
TBS	The Bethesda system
UCSF	University of California–San Francisco
USPSTF	United States Preventive Services Task Force

Specimen Adequacy

1

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Adequacy Categories

Satisfactory

Satisfactory for evaluation

(describe presence or absence of endocervical/transformation zone component and any other quality indicators, e.g., partially obscuring blood, inflammation, etc., as appropriate)

Unsatisfactory

For unsatisfactory specimens, indicate whether or not the laboratory has processed/evaluated the slide. Suggested wording:

A. *Rejected specimen:*
Specimen rejected (not processed) because _____ (specimen not labeled, slide broken, etc.)
B. *Fully evaluated, unsatisfactory specimen:*
Specimen processed and examined but unsatisfactory for evaluation of epithelial abnormality because of _____ (obscuring blood, etc.)

Additional comments/recommendations, as appropriate

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1.1 Background

Evaluation of specimen adequacy is considered by many to be the single most important quality assurance component of the Bethesda system. The first two versions of the Bethesda terminology included three categories of adequacy: satisfactory, unsatisfactory, and a "borderline" category initially termed "less than optimal" and then renamed "satisfactory but limited by" in 1991. The 2001 Bethesda system eliminated the borderline category, in part, because of confusion among clinicians as to the appropriate follow-up for such findings and also due to the variability in criteria used to report "satisfactory but limited by" among laboratories [1]. To provide a clearer indication of adequacy, specimens are now designated as either "satisfactory" or "unsatisfactory."

Prior to the 2001 Bethesda system (TBS), criteria for determining adequacy were based entirely on expert opinion and the few available studies in the literature. Laboratory implementation of some of these criteria was shown to be poorly reproducible [2–4]. In addition, the increasing use of liquid-based cytology necessitated developing criteria applicable to these preparations. The 2001 Bethesda adequacy criteria were based on published data to the extent possible and were tailored to both conventional and liquid-based preparations. For this edition of the TBS atlas, data and clinical experience regarding specimen adequacy since 2001 were reviewed, leading to the offering of additional guidance for special situations, such as assessing cellularity in specimens obtained from postradiation patients, interfering substances and human papillomavirus testing.

1.1.1 Explanatory Notes

For satisfactory specimens, information on transformation zone sampling and other adequacy qualifiers should also be included in the report. Providing clinicians/specimen takers with regular feedback on specimen quality promotes heightened attention to specimen collection with consideration for the use of improved sampling devices and preparation technologies.

Any specimen with abnormal cells (atypical squamous cells of undetermined significance (ASC-US), atypical glandular cells (AGC), or worse) is by definition satisfactory for evaluation. If there is concern that the specimen is compromised, a note may be appended indicating that a more severe abnormality cannot be excluded.

Unsatisfactory specimens that are processed and evaluated require considerable time and effort on the part of the laboratory. Although an epithelial abnormality cannot be excluded in such specimens, reporting of information such as the presence of organisms, or endometrial cells in women 45 years of age or older, etc. (see Chap. 3), may help direct further patient management [5]. Note that the presence of benign endometrial cells at any age does not make an otherwise unsatisfactory specimen satisfactory.

Longitudinal studies looking at both conventional and liquid-based preparations found that unsatisfactory specimens that were processed and evaluated were more often from high-risk patients, and a significantly greater number of these were followed by a squamous intraepithelial lesion (SIL) or cancer when compared to a cohort of satisfactory index specimens [6–8]. Unsatisfactory cases which are hrHPV positive have been reported to have a much higher risk for precancerous lesions than those that are hrHPV negative [8].

1.2 Minimum Squamous Cellularity Criteria

1.2.1 Cellularity

There is no further evidence since the last Bethesda System update in 2001, to support adjustment of the minimum cellularity requirements for routine cervical cytology screening and follow-up. However, published literature and laboratory practice experience since the 2001 Bethesda workshop demonstrates ongoing confusion regarding the minimum cellularity estimates in special circumstances. Cytologists have often applied rigid minimum cellularity estimates to vaginal and postradiation or post-chemotherapy specimens, leading to a high unsatisfactory rate in these settings [9]. Quiroga-Garza found that almost half of 276 women with unsatisfactory results were over 50, and 85 % of these women had a history of gynecologic cancer. The most common cause for the unsatisfactory specimens was low squamous cellularity [10]. Women who have received radiation, chemotherapy, hysterectomy, or trachelectomy for invasive cancer often develop atrophic and reparative cellular changes, and when a cervix remains, there is frequently stenosis and altered anatomy [11]. There is little scientific evidence that a minimum cell threshold of 5,000 is required in these circumstances; some investigators recommend a lower threshold of 2,000 cells in these patients [12]. The 2001 Bethesda atlas stated that minimum cellularity criteria were developed for use with all cervical cytology specimens, but it is emphasized in this update that a 5,000 cell threshold should not be rigidly applied in vaginal and post-therapy specimens.

Liquid-Based Preparations (Figs. 1.1–1.11):

An adequate liquid-based preparation (LBP) from a woman with a cervix should have an estimated minimum of at least 5,000 well-visualized/well-preserved squamous or squamous metaplastic cells. This range applies only to squamous cells. Endocervical cells and completely obscured cells should be excluded from the estimate. Women who have had chemo- or radiation therapy, who are postmenopausal with atrophic changes, or who are post-hysterectomy may have samples with fewer than 5,000 cells, and such specimens may still be considered adequate at the discretion of the laboratory. The patient history must be taken into consideration in such cases. Samples with less than 2,000 cells, however, should be considered unsatisfactory in most circumstances.

Some have advocated that LBPs with 5,000–20,000 cells are of borderline or low squamous cellularity. In specimens with suspected low cellularity, an estimation of total cellularity can be obtained by performing representative field cell counts. A minimum of ten microscopic fields, usually at 40×, should be assessed along a diameter that includes the center of the preparation and the average number of cells per field estimated. When there are holes or empty areas on the preparation, the percentage of the hypocellular areas should be estimated, and the fields counted should reflect this proportion. Although both LBPs have similar numbers of cells overall, SurePath™ (BD Diagnostics, Durham, NC) slides have a higher cell density than do ThinPrep™ (Hologic, Inc., Bedford, MA) slides because of the smaller preparation diameter with SurePath™ (see Table 1.1). Siebers et al. evaluated several different protocols for estimation of low cellularity ThinPrep™ specimens and found that counting five fields along a horizontal diameter and five fields along a vertical diameter (SKML protocol) at 10× had the best correlation with a reference method that utilized image analysis software for counting cells [13]. However, when all of their measurements at different objective powers were merged, the differences between the SKML and the Bethesda protocols (as noted above) were not statistically significant.

Table 1.1 provides the average number of cells per field required to achieve a minimum of 5,000 cells on an LBP given the preparation diameter and field number of the eyepiece (ocular). For individuals using eyepieces and preparations not shown, the formula is: number of cells required per field = 5,000/(area of preparation/area of field). The diameters of SurePath and ThinPrep preparations are 13 and 20 millimeters (mm), respectively. The diameter of a microscopic field in millimeters is the field number of the eyepiece divided by the magnification of the objective. The area of the field is then determined by the formula used to calculate the area of a circle [pi × radius squared, πr^2]. The magnification power of the ocular does not affect this calculation [14, 15]. For additional explanation of the pertinent optical principles, see http://www.microscopyu.com/articles/formulas/formulasfieldofview.html.

Figures 1.1, 1.2, 1.3, 1.4, and 1.5 show cell coverage or density in unsatisfactory, borderline satisfactory, and satisfactory liquid-based preparations. These are *not* reference images, as they do not represent an entire microscopic field; thus, the cell density shown in the images cannot be compared directly to Table 1.1 for estimation of squamous cellularity.

In some instances, the cellularity on the prepared slide may not be representative of the collected sample. Slides with fewer than 5,000 cells should be examined to determine if the reason for the scant cellularity is a technical problem related to slide preparation such as an excessively bloody specimen. If a technical problem is identified and corrected, a repeat preparation may yield adequate cellularity (Fig. 1.6a, b). However, the adequacy of each slide should be determined separately and not cumulatively. Attempts to determine cellularity cumulatively by summing the cellularity of multiple inadequate slides may be confounded by uncertainty regarding the true cellularity of the specimen (not the slide), which might be substantially less than in a specimen with normal slide cellularity. This matter is in need of more research, and hence this guideline may be subject to change in the future. Given the relatively low minimum criterion for adequate cellularity, caution is warranted in borderline cases. The report should clarify whether blood, mucus, lubricant, inflammation, or technical artifact contributed to an unsatisfactory sample or whether the problem was simply low squamous cellularity.

Table 1.1 Guidelines for estimating cellularity of liquid-based preparations

		FN20 eyepiece/10× objective		FN20 eyepiece/40× objective		FN22 eyepiece/10× objective		FN22 eyepiece/40× objective	
Prep. diameter (mm)	Area (mm^2)	Number of fields at FN20, 10×	Number of cells/field for 5K total	Number of fields at FN20, 40×	Number of cells/field for 5K total	Number of fields at FN22, 10×	Number of cells/field for 5K total	Number of fields at FN22, 40×	Number of cells/field for 5K total
13	132.7	42.3	118.3	676	7.4	34.9	143.2	559	9.0
20	314.2	100	50.0	1,600	3.1	82.6	60.5	1,322	3.8

FN field number