AMERICAN SOCIETY OF CYTOPATHOLOGY

CERVICAL CYTOLOGY PRACTICE GUIDELINE

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I. INTRODUCTION

I.A. Purpose, Intended Audience, and Limitations

"Clinical practice guidelines are systematically developed statements to assist practitioner and patient decisions about appropriate health care for specific clinical circumstances." [1, 2]
Practice guidelines are used by diverse segments of the medical community to define and communicate standards of performance and care. To date, the American Medical Association has catalogued nearly 2,000 clinical practice guidelines or equivalent documents.[3] An additional source on practice Guidelines is the web site www.guideline.gov (National Guideline Clearinghouse) that is sponsored by the Agency for Health Research and Quality (AHRQ) formally the Agency for Health Care Policy and Research in partnership with the American Medical Association and the American Association of Health Plans.

The Cervical Cytology Practice Guideline is a document for laboratories and is intended for use primarily by cytologists – pathologists and cytotechnologists – who perform cervical cytology analyses and report their findings to clinicians. Thus, this document focuses on laboratory processes and related topics such as techniques of sample procurement, slide staining and analysis, and cytology laboratory management. This guideline is intended for use by laboratorians; however, clinicians, patients, and others involved in women's healthcare will find this document to be a resource in making clinical care decisions.

The process of creating Guideline represents consensus building within a specialty with subsequent endorsement by national professional organizations. In light of rapidly evolving science and technology, a guideline devoted to cervical cytology requires timely review and revision. This guideline serves not as a specific blueprint or set of dictates, but as a device to assist standardization and continuous quality improvement efforts. It is with this understanding that the American Society of Cytopathology promulgates this Cervical Cytology Practice Guideline.

I.B. Context and Scope

The emphasis of this guideline is on cervical cytology specimen procurement, analysis, reporting and management. Specific microscopic criteria for interpretation are not included since these have been well described in textbooks, symposia and workshops. A detailed analysis of related clinical topics such as patient care algorithms for follow up of abnormal cervical cytology results, are also beyond the scope of this document.

An important general limitation is that this guideline, in many respects, is applicable for laboratories in the United States only. Many of its elements are defined or specified in United States government agency regulations.

I.C. Variability of Practice

This document highlights procedural and interpretive areas where there are variations in practices, and areas where there is consensus for "best practices". Where the literature is conflicting, absent, or consists only of case reports rather than more comprehensive studies, this document describes different laboratory practices.

II. Epidemiology and Public Health

II.A. Incidence and Mortality

Cervical cancer mortality has decreased 70% over five decades, largely attributable to the introduction of cervical cytology screening in the 1940's. Cervical cancer, once a leading cause of cancer death in women in the US, now ranks 13th. An estimated 12,800 women are still diagnosed each year with invasive cervical cancer and approximately 4,600 will die of their disease.[4] However, worldwide, cervical cancer is the second most common cancer in women (following breast cancer); it ranks first in many developing countries lacking screening programs.[5]

Cervical cytology screening targets squamous cell carcinoma, although epidemiological data includes statistics for all subtypes of cervical malignancy.[4] Squamous cervical carcinoma is an ideal target for screening. The cervix is accessible, associated with low sampling morbidity, and therapeutic intervention is effective during the relatively slow development from precursor lesions to invasive cancer. Cervical neoplasia typically develops over 10 years prior to becoming invasive. Although "rapidly progressing" forms of invasive carcinoma of the cervix have been postulated, there is no firm evidence to suggest that the natural history of invasive carcinoma is changing. Upon detailed review, a number of cases of "rapidly progressive" cervical cancer can be ascribed, at least with the advantage of hindsight, to screening failure.[6, 7, 8, 9]

Scandinavian studies demonstrate most convincingly the value of screening Pap smears. Studies have shown those countries with formal screening programs and wide population coverage experienced substantial drops in incidence and mortality while neighboring countries with limited population screening did not.[10, 11]

The success of cervical cytology screening lies in its relative simplicity, low cost and noninvasive nature. Annual screening reduces the probability of developing invasive carcinoma by over 95%. Most cases of invasive cervical carcinoma occur because a patient is not screened, not screened at an appropriate interval, or there is inadequate follow up for an identified abnormality.[12]

II.B. Risk Factors for Cervical Cancer

II.B.1. Human Papillomavirus (HPV)

The pathogenesis of cervical neoplasia and cervical cancer is related to HPV, based on epidemiological, virological, and experimental evidence.[13, 14] Most previously identified risk

factors for cervical cancer such as early age of first intercourse and increased number of sexual partners, reflect risk of exposure to HPV.

There are more than 80 types of human papillomaviruses (HPVs) including some that cause the common warts that grow on hands and feet. Approximately 30 types have the ability to infect the anogenital tract and can be passed from one person to another through sexual intercourse. About 15 genital HPVs have been found in cervical cancer and are termed "cancer-associated" types. HPV 16 is the most important type associated with cancer in almost all geographic regions, along with HPVs 18, 31, and 45. Genital warts, known as condylomata acuminata, are generally associated with low-risk HPV types 6 and 11.

HPV infects reproducing cells; infection of the cervix occurs at the basal cell layer. HPV is a double stranded DNA virus that has three well studied regions: an upstream regulatory region (URR) gene that does not code proteins, early genes (E) which code for nonstructural proteins, and late (L) genes which code for structural proteins such as the viral capsid. When HPV infects a basal cell and is not integrated into the host genome, the viral DNA replicates within the host cell and remains within the cell as it grows toward the surface layers. The early genes are tightly regulated by E2, which suppresses the action of the oncogenes E6 and E7. When L genes are subsequently activated, entire encapsulated virions are produced which are expressed morphologically as "koilocytes." When HPV is integrated into the host DNA, there is often disruption of the E2 regulatory gene. Loss of regulation leads to expression of E6 and E7, leading to cell proliferation. This proliferating cell population is at risk for transformation to high grade lesions or carcinoma. [15, 16, 17]

HPV infection is very common while cervical cancer is not. Host and environmental factors are postulated to influence the risk of progression from HPV infection to cancer precursors and invasive cancer.

Immunodeficiency is associated with higher rates of HPV infection and progression. HIV infection, particularly in women with low CD4 counts, is associated with a high prevalence of HPV DNA and SIL detection.[18, 19, 20] An increased incidence of HPV is also associated with other immunosuppressed states, such as organ transplant recipients, chronic renal failure, a history of Hodgkin[21] lymphoma and immunosuppressive therapy.[22]

Currently, there is no consensus in the medical literature supporting routine HPV testing as part of cervical screening. There is a growing body of literature suggesting that HPV testing may be an option in the management of patients with equivocal abnormal results, however, this practice is not yet widely adopted. A large-scale clinical trial [the ASCUS/LSIL Triage Study (ALTS)] evaluating the cost-effectiveness of this approach, among other management strategies, is awaiting completion.[23]

II.B.2. Other Co-factors

Oral contraceptive use has also been associated with a 1.5 fold relative increased risk for developing cervical carcinoma. Although the increased risk has been ascribed to the lack of barrier

type contraception (and therefore more exposure to HPV), controlled studies have found a relative increased risk suggesting a separate causal relationship.[24, 25, 26]

When controlled for confounding risk factors, cigarette smoking has been cited as an independent risk factor for the development of cervical carcinoma. Smokers were found to have a 50% higher risk for developing cervical carcinoma than nonsmokers. Risk increases with the increased number of cigarettes, duration of smoking history and use of unfiltered tobacco products.[27, 28]

II.B.3. Screening

Lack of cervical cytology screening is a significant risk factor for cervical cancer. Previous population studies suggested that African-Americans, Hispanics and Native Americans were considered at greater risk for cervical carcinoma in the United States. However, when corrected for screening coverage, race, as a risk factor is noncontributory.[29]

Historically, unscreened populations of women in the U.S. have included older women, uninsured and impoverished women, minority women – particularly Hispanic and older African-American women, and women residing in rural areas.[30] Recent surveys indicate that many of these patterns remain unchanged despite increased screening efforts. In the 1994 National Health Interview Survey of the U.S. population, 77% of women reported having had a Pap test in the past three years. Age remains a factor; screening was higher among women 18-44 (82%) compared to women 65 and older (57%). However, there were no marked differences between African-Americans, Hispanic whites, and non-Hispanic whites, or metropolitan versus non-metropolitan residents in the 18-44 year old age group. Socioeconomic measures continue to show significant differences in screening coverage. Women who did not complete high school and whose family income was less than $20,000 reported lower rates of screening compared to women with education beyond high school or family income exceeding $20,000.[31] Lack of access to health care, lack of routine examinations, lower education level and risk perception ("beliefs") are barriers to cervical cytology screening that are reflected in socioeconomic status.[32] When barriers to cervical cytology screening are removed, the incidence of invasive carcinoma declines in the population.[33]

Regular cytologic screening for cervical cancer reduces both the mortality and incidence of cervical carcinoma in the screened population. Annual cytological screening will reduce the incidence of invasive squamous carcinoma by more than 95%.[12] Despite acknowledgement that routine screening Pap smears are effective, the interval of routine screening remains controversial. Various organizations have different recommendations for interval screening. The United States Preventive Services Task Force recommends cervical cytology screening every 3 years for sexually active women with an intact cervix.[34] The American Cancer Society (ACS) suggests that annual screening cytology should be performed on all sexually active women until three adequate negative smears are obtained. The interval between subsequent screening is at the physician's discretion.[35] The American College of Obstetricians and Gynecologists' recommendations are similar to the ACS, but more frequent continual screening of high-risk women is encouraged.[36] The American College of Physicians recommends cervicovaginal cytology screening every three years between age 20 to 65.[37] The College of American Pathologists advocates that, in general, all women who are, or have been sexually active, or who have reached 18 years of age, should have an annual cervical cytologic examination (Pap test) and pelvic examination.[38] Despite the

variety of interval recommendations, many physicians continue to perform annual screening in the US, which the American Society of Cytopathology endorses.

III. Specimen Collection and Submission

The importance of proper specimen collection and submission cannot be overemphasized. At least one half to two thirds of false negatives are the result of patient conditions present at the time of sample collection and submission and the skill and knowledge of the individual who obtains the specimen.[39, 40, 41] The clinical community is responsible for training health care personnel to assure that adequate cervical cytology samples are collected and submitted to the laboratory with appropriate clinical information. The laboratory provides feedback on sample adequacy via individual reports, and may elect to provide summary information regarding patient sampling to its clients.

III.A. Patient Preparation

To optimize collection conditions, a woman should: [42]

1. Schedule an appointment approximately two weeks (10-18 days) after the first day of her last menstrual period.
2. Not douche 48 hours prior to the test.
3. Not use tampons, birth control foams, jellies or other vaginal creams or vaginal medications for 48 hours prior to the test.
4. Refrain from intercourse 48 hours prior to the test.

III.B. Test Requisition

Under the supervision and guidance of the physician, a laboratory requisition must be legibly and accurately filled out before obtaining the cellular sample. The laboratory requisition is the main communication link between the physician and the laboratory. The requisition should request the following information as required by CLIA ’88.[43]

1. Patient’s name (any name change in the past 5 years should be noted.)
2. Age and/or date of birth.
3. Menstrual status (LMP, hysterectomy, pregnant, postpartum, hormone therapy.)
4. Previous abnormal cervical cytology result, previous treatment, biopsy or surgical procedure.
5. Patient’s risk status for developing cervical cancer, e.g. “high risk”. The clinician should expect that the laboratory would rely upon the information provided on the current requisition in arriving at an assessment of risk status. (See section IIB.)
6. Source of specimen, e.g. cervical, vaginal.

Appropriate clinical history provided by the physician on the requisition should include:

1. Hormone/contraceptive use.
2. Relevant clinical findings (abnormal bleeding, grossly visible lesion, etc.)

III.C. Labeling the Sample

The glass slide or specimen vial must be labeled with a unique identifier, usually the patient's first and last names, at the time of the collection of the cellular sample. Individual laboratories may require a second identifier such as date of birth, medical record number, social security number or collection date. The lab must have a written procedure that specifies the requirements for proper specimen identification. For glass slides, the required information is written in solvent resistant pen or pencil on the frosted end of the slide. For liquid based samples, the required information must be affixed to the vial.

III.D. Visualization of the Cervix for Collection of an Adequate Sample

Collection of a cervical cytology specimen is usually performed with the patient in the dorsolithotomy position. A sterile, or single-use bivalve speculum of appropriate size is inserted into the vagina without lubrication. Warm water may be used to facilitate insertion of the speculum. The position of the speculum should allow for complete visualization of the os and ectocervix.

The transformation zone is the site of origin for most cervical neoplasia and should be the focus of cytology specimen collection.[44] The transformation zone may be easily visualized or may be high in the endocervical canal. Location varies not only from patient to patient, but in an individual over time. Factors producing variation include changes in vaginal pH, hormonal changes including pregnancy, childbirth, and menopausal status, and hormonal therapy. In postmenopausal patients or women who have received radiation therapy, cervical stenosis may prevent visualization of the transformation zone. It remains important to sample the endocervix in these patients. This may require more extensive clinical procedures. If a patient has had a hysterectomy, a vaginal sample is sufficient, with particular attention to sampling the vaginal cuff.

III.E. Collection Devices

There are a variety of collection devices available for sampling the endocervix, transformation zone and ectocervix. They include endocervical brushes, wooden and plastic spatulas, and plastic "broom-type" samplers. Plastic spatulas are preferred over wooden since the wooden spatulas retain cellular material.[45] The use of a cotton-tipped swab is NOT recommended, even if the swab is moistened.[41] Cells adhere to the cotton and do not transfer well to the glass slide, which results in an incomplete specimen. Analysis of different sampling methods has shown that overall, the cytobrush and spatula together provide the best specimen for cervical cytology .[46] However, the choice of a particular device is dependent on variations in the size and shape of the cervix and the clinical situation. As stated in III.D., age, parity, and hormonal status of the patient can affect the exposure of the transformation zone. Previous therapy, such as conization, laser therapy or cryotherapy, can also change the features of the cervix. The clinician ought to consider these factors when choosing a collection device.[41] Liquid based methods require the use of collection devices that have been approved by the FDA for use with the particular specimen preparation instrument.

III.F. Techniques for Sample Collection

III.F.1. Collection of cervical/vaginal specimens for conventional smear preparation using the spatula and endocervical brush

The vaginal fornix and ectocervix should be sampled before the endocervix/transformation zone. First, a sample of the ectocervix is taken using a plastic (or wooden) spatula. The notched end of the spatula that corresponds to the contour of the cervix is rotated 360° around the circumference of the cervical os, retaining the sample on the upper surface of the spatula. Grossly visible lesions, including irregular, discolored or friable areas should be directly sampled and can be placed on a separate slide, especially if the lesion is distant from other collection areas. The spatula is held with the specimen face up while the endocervical sample is collected.

Sampling of the endocervix requires insertion of the endocervical brush into the endocervical canal until only the bristles closest to the hand are visible. The brush is rotated 45-90° and removed. At this time, the sample on the spatula is spread evenly and thinly lengthwise down one half of the labeled slide surface, using a single uniform motion. The endocervical brush is then rolled along the remaining half of the labeled slide surface by turning the brush handle and slightly bending the bristles with gentle pressure. The brush should not be smeared with force or in multiple directions.[41, 47] The entire slide is then rapidly fixed by immersion or spray and the collection devices are discarded. Note: use of the endocervical brush may be contraindicated in pregnant patients. Refer to the package insert. If the above-described sampling order is reversed, bleeding secondary to abrasion from the brush may obscure the cellular material.

III.F.2. Collection of cervical/vaginal specimens for liquid-based preparations using the spatula and endocervical brush

For liquid based preparations, the ectocervix should be sampled using the same procedure as for conventional Pap smears. However, the spatula with the cellular material is rinsed in the specimen vial and then discarded. The endocervical specimen is collected using the same technique as for conventional Pap smears. However, the endocervical brush is rinsed in the vial and then discarded. Manufacturers' directions must be followed.[48]

III.F.3. Collection of cervical/vaginal specimens for conventional smear preparation using the "broom-like" device

The ectocervix and endocervix are collected simultaneously with the "broom-like" device. The central bristles of the broom are inserted into the endocervical canal until the lateral bristles bend fully against the ectocervix. The sampling device is rotated 360º in the same direction five (5) times while maintaining gentle pressure. The broom is removed and with a single paint stroke motion the cellular sample is transferred down the long axis of the labeled surface of the slide. The broom is turned over and the paint stroke motion is repeated over the same area. The slide is rapidly fixed either by immersion or spray and the device is then discarded.

III.F.4. Collection of cervical/vaginal specimens for liquid-based preparations using the "broom-like" device

The ectocervical and endocervical specimens are collected with the "broom-like" device simultaneously. The central bristles of the device are inserted into the endocervical canal until the lateral bristles fully bend against the ectocervix. Maintaining gentle pressure, the broom is rotated

in a clockwise direction 360º for a total of five (5) times. The broom is then rinsed in the specimen vial. Manufacturers' directions vary and must be referred to and followed.[48, 49]

III.G. Cell Fixation for Conventional Cervical Cytology

Immediate fixation of the cellular sample, within seconds of specimen collection, is necessary to prevent air-drying. Air-drying obscures cellular detail and compromises specimen evaluation. Immersing the slide in alcohol or spraying with fixative can prevent air-drying artifact.

If the specimen is immersed in alcohol, it may remain in the alcohol for transport to the laboratory. Alternatively, the specimen can be immersed in alcohol for 20-30 minutes, removed and allowed to air dry, then placed in a container/mailer for transport to the laboratory.[50] The immersion technique requires use of a separate container for each specimen and changing or filtering the alcohol between specimens.

If a specimen is spray fixed, only quality controlled cytology fixatives should be used. Hair spray should NOT be used. Whether using a pump spray, aerosol fixative or single application packet, the manufacturer's instructions on the container and package insert should be followed. Generally, spray fixatives should be 6-10 inches (15-25 cm) from the glass slide when applied.[41]

III.H. Variability in Specimen Collection and Submission Practices

Variations in specimen collection include the use of conventional Pap smear collection on a glass slide/slides or collection in a liquid fixative. Additional variation is encountered in rinsing the collection devices and handling of the devices after the specimen has been collected. Manufacturers' instructions and/or package inserts should be consulted and recommendations followed.

Other variations include the use of different collection devices. The plastic spatula is preferred to the wooden spatula. The endocervical brush is preferred for sampling of the endocervix. The "broom-like" device is also available. Clinical judgment is required to determine the appropriate device, as there is no single sampling device that is optimal for all clinical circumstances.

There is variation in placement of the vaginal, ectocervical and endocervical samples on the glass slide. For VCE slides, the vaginal sample is collected first and placed on the slide near the frosted end within the section labeled "V". The ectocervical specimen is then collected and smeared within the section of the slide labeled "C". The endocervical specimen is collected last, and smeared within the section of the slide labeled "E". The slide is then rapidly fixed. Another option is to mix a vaginal pool sample with the cervical specimen. This somewhat protects the cellular material from air-drying prior to fixation. Yet another option is to smear the ectocervical specimen on the slide, and then directly roll the endocervical brush on top followed by fixation.

No consensus has been reached on the clinical benefit of one slide versus two slides for cervical cytology. Several comparative studies have been performed and concluded that the single slide

method is an acceptable alternative to the double slide method. The single slide method decreases the number of slides screened in the laboratory, reduces costs for glass slides, and requires less space for storage.[51, 52]

While this section discusses the consensus of the cytologic community regarding the most appropriate and effective methods of specimen collection and submission, it is not intended to supplant or establish the gynecologic community's standard of care and practice regarding these issues. Nor is this Guideline intended to diminish the responsibility of clinicians to be aware of and apply the standards applicable to their medical specialty and their individual patients.

IV. Laboratory Sample Processing

Laboratory sample processing includes steps from the receipt of the specimen in the laboratory to the delivery of a stained slide ready for microscopic examination. The information is based upon practices cited in standard cytology references.[53, 54, 55, 56] Throughout processing, the integrity of the specimen must be maintained [57] and the principles of universal precautions followed.[58] No result is to be released unless the system is functioning properly.

IV.A. Receipt and Identification of the Specimen

The laboratory should confirm the integrity of the specimen received. Specimens are accepted only when ordered by physicians or other persons authorized by law. To process, each sample must have an accompanying request form completed by the authorized provider. The laboratory should have a procedure in place for handling oral requests. The provider must properly label specimens.

IV.A.1. Requisition Requirements

The requisition accompanying the specimen should be completed with the patient's first and last name and the age or date of birth at a minimum.[43] The date the sample was collected, the source of the material and the name, location and telephone/FAX number of the requesting physician should be included on the requisition. A medical record number or any other unique identifier may also be included. These elements are required to ensure that specimen results are linked with the appropriate patient. They also make it possible for the laboratory to make prior and/or concurrent results available at the time of cytologic interpretation if necessary.

Ideally, the following information should also be provided on the requisition form as applicable: LMP, pregnant, postmenopausal, estrogen therapy, other hormonal therapy, IUD, DES exposure, chemotherapy, radiation therapy, GYN surgeries, history of cancer, previous abnormal cervical cytology, clinical findings such as infection or a grossly visible lesion and any factors that place the patient at increased risk for developing cervical cancer.

Clinical history is important and should be correlated with the type of specimen submitted. For example, if the history states that the patient has had a total hysterectomy and the specimen is a cervical sample, clarification and resolution of the discordance should be undertaken before interpretation of the slide(s) is attempted. All available patient information should be included in the demographic and clinical history sections of the report and archived database for current and future use.

A written procedure must be in place to handle specimens that are received without adequate information on the request form.

IV.A.2. Glass Slides

Written criteria for the rejection of specimens must be available in each laboratory and should address unlabeled slides, slides labeled with non-permanent writing utensils or paper labels, broken slides, and slides with any piece of the cellular portion missing. Any slides that are broken beyond repair should not be accepted. The submitting clinician should be notified and the

notification documented in the laboratory. For slides that can be repaired, a comment regarding the sample condition should be noted in the report.

IV.A.3. Liquid Based Specimens
The specimen vial should be received tightly closed with no leakage of the preservative and with patient identification on the vial (not the lid). If the preservative has leaked into the transport container, this should be documented and every reasonable effort should be made to salvage the sample. However, if an excessive amount of the preservative has been lost, the specimen may not be sufficient for evaluation; in which case, the clinician should be notified and the notification documented in the laboratory.

IV.B. Accessioning

When the specimen and requisition are removed from the transport container, the specimen identifiers on the requisition form and sample must match. Any variation in the spelling of the name or in the medical record number or other unique identifier should be questioned and verified. The requesting physician or designee may rectify variations; the laboratory must keep a record of all changes made, according to the lab's standard operating procedure. When all specimen identifiers match, the specimen is accessioned; that is, assigned a unique number which identifies this specimen as belonging to this patient. The number may be generated manually or electronically. This unique number is placed on the slide and on the requisition using a material or marking device such that the number will withstand subsequent processing. Following staining and coverslipping, a label may be affixed over a handwritten name and number.

IV.C. Staining

IV.C.1. Smears
Any slides fixed with spray fixatives that contain Carbowax should be soaked in ethanol or water before beginning the staining process. Carbowax is a water-soluble substance that is removed with soaking. Carbowax left on the slides will impede stain uptake.

IV.C.2. Liquid Based Specimens
Liquid based specimens should be processed according to the manufacturer’s instructions for transfer of cells from the liquid medium to a glass slide labeled with the patient's name and accession number. A written procedure should be in place for rejection of liquid based specimens that are not collected following the manufacturer's guideline. Refer to additional discussion in section IX, “Enhancements to Cervical Cytology Testing”.

IV.C.3. Staining Procedure
The modified Papanicolaou method is recommended for the staining of gynecologic cytology slides.[44, 55, 59] The Papanicolaou method uses a standard nuclear stain, hematoxylin, and two cytoplasmic counterstains, OG-6 and EA. The value of this method is transparency of the cytoplasm, which allows the examiner to clearly visualize cellular morphology. Either a progressive or regressive technique may be used for nuclear staining. Several automatic

programmable stainers are available. Each laboratory should develop a staining protocol for manual, automated, or for both methods, which results in the optimum staining of the specimen.

Maintenance of consistently good staining requires that the stains are filtered and changed on a regular schedule, determined either by the number of slides processed or the length of time elapsed since stains were last changed. Furthermore, the quality of the stain should be monitored daily and the results documented. Deviations from optimum quality should be addressed immediately, the problem identified and corrective action(s) taken. The laboratory must document all problems and corrective action taken. If the stain quality is acceptable, the remaining smears are stained and submitted for screening.

To prevent cross-contamination, gynecologic smears are usually stained separately from non-gynecologic smears. If a single staining setup is used, solutions should be changed or filtered between gynecologic and non-gynecologic specimens. In any staining configuration, samples with a high potential for cross-contamination must be stained separately from the remainder of the laboratory's cases.[60, 61]

IV.D. Dehydration, Clearing and Coverslipping

IV.D.1. Dehydration and Clearing

After staining, the sample is dehydrated using a series of increasing concentrations of alcohol followed by baths in clearing solutions. The last must be colorless and its refractive index must be close to that of the coverslip, slides and mounting medium. While xylene (dimethyl-benzene) is the most commonly used clearing agent, others derived from citrus terpenes and other sources have found some use. If using xylene, clearing should be performed in a well-ventilated area or fume hood to limit exposure to xylene fumes.[62] Slides should remain in the clearing agent until coverslipping is performed.

IV.D.2. Coverslipping

Mounting medium used to bond the slide and the coverslip must be compatible with the clearing agent, must be transparent, and should have a refractive index that is similar to the glass slide and the specimen. The boundaries of chromatin particles are the most distinct when the specimen is mounted in a medium of similar refractive index. Glass slides according to the American Society for Testing and Materials (ASTM) [63] specifications have a refractive index of 1.515. The refractive index of cells is similar to that of glass. Most commercially available mounting media have refractive indices that range from 1.49-1.57+. Mountants that exceed this range should not be used. Ideally, the refractive index should be 1.52-1.54.

Adequate mounting medium should be applied to protect the cellular material from air-drying and shrinkage, and to form a protective seal to prevent fading of the cell sample. The cellular material should be completely covered by a suitably sized coverslip or covering material of appropriate quality. The ASTM requires that coverslips have a refractive index of 1.523$\pm$.005. Microscope manufacturers recommend a total thickness of mountant and coverslip between 0.17 and 0.18mm. Therefore, No. 1 coverslips (0.13-0.17mm) should be used.

Coverslipping requires good light, ventilation and eye protection. Slides should be removed from xylene one at a time to avoid drying of the cell surface. Different methods used to coverslip include placing the mounting medium on the coverslip, then inverting the coverslip onto the slide surface, or lowering the slide onto a coverslip containing adequate mounting medium. Glass coverslips, coverfilm and automated coverslippers are available. The mounting medium should be allowed to dry before the slides are reviewed to reduce movement of cellular material during the slide examination.

Chemical waste collected throughout the staining, dehydration, clearing and coverslipping processes should be disposed of according to the OSHA Guideline.[62]

IV.E. Destaining and Restaining

Destaining a slide is a stepwise process, beginning with removal of the coverslip and mounting medium, and proceeding backward through the staining steps, omitting the stains themselves. Alternatively, once the coverslip and mountant are removed the slide can be soaked in acid alcohol until the slide is colorless. The process is completed by thoroughly rinsing the slide in water baths. Once destaining is complete, restaining can begin at the nuclear stain step.[56]

IV.F. Collation of Slides and Requisitions

The stained and labeled slide should be matched with its requisition or other laboratory document that displays the same information. The information on the slide must correspond to the information on the requisition or lab document. If there are any discrepancies, this must be noted and resolved BEFORE the report is released.

IV.G. Configuration of Laboratory Space According to Function

The laboratory must have adequate space to ensure that the quality of preparatory work, interpretive services and the safety of laboratory personnel are not compromised.[58, 61, 64, 65, 66]

IV.H. Variability in Practice

The criteria for accepting/rejecting specimens vary among laboratories. Minimum requirements for patient information differ as well. See section VI.A. for more specific examples of clinical information.

There are several methods used for handling broken slides when a piece of the cellular portion is missing. Some laboratories will not process the sample; others report the slide as "Satisfactory but limited by…" and comment on the condition of the smear when it was received.

There are currently two different FDA approved methods to collect and process liquid-based specimens. See also section IX. The protocols are not interchangeable; therefore, the manufacturer's Guideline in the operator’s manual of the method chosen must be followed.

Accessioning specimens can be performed with a hard copy of the patient requisition or requisitions can be received electronically.

Sixty millimeter coverslips are recommended for conventional Pap smears as they consistently cover the entire smeared area. Shorter coverslips are acceptable for conventional smears and for liquid based preparations as long as the cellular material is covered.

V. Cervical Cytology Analysis

V.A. Individual Qualifications

Individuals qualified according to the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88) must perform analysis of cervical cytology specimens.[67] In most laboratories, screening is performed by cytotechnologists. Adequate support personnel should be available to minimize clerical duties for cytotechnologists. The laboratory must have a qualified pathologist serving as laboratory director or technical supervisor, and a general supervisor as defined by CLIA 88.[68]

Additional training is required to screen liquid-based cytology specimens and to perform computer-aided slide examination.

V.B. Environment and Equipment

Examination of cervical cytology slides should be performed in a comfortable area of the laboratory with minimal distractions. Ergonomics play a vital role in the cytotechnologist's workstation to minimize the risk of repetitive motion injury and musculoskeletal strain. Adequate space, facilities and equipment must be made available to the cytotechnologist to perform his or her duties. Regular monitoring and maintenance of all equipment and instruments is essential. Proper equipment and resources include: sufficient desk or bench space, a cushioned chair with seat and height adjustment as well as adjustable back support, and a microscope in good working order. Arm rests that fit the desktop, tilting microscope heads, rubber focus knob adapters and devices that adjust microscope height are available options that increase the comfort of the technologist. Other factors include diffuse, moderate room illumination, a non-reflective desk surface, and a comfortable, draft-free room separate from the processing area [58, 61] where protective equipment is required. Clerical and record-keeping areas of the laboratory should be located near the screening area.[66]

V.C. Analysis Time

The actual amount of time spent analyzing a given slide is highly variable. Factors influencing the amount of time spent examining a cervical cytology slide include method of sample preparation (liquid based vs. conventional), overall sample cellularity, blood, inflammation or other obscuring factors, clinical history, complexity of findings and the cytologist's experience and state of mind. Workload limits must be set for each individual based upon an evaluation of the individual cytologist's capability and, where applicable, feedback provided by the cytologist in the evaluation process, and must not exceed the limits set by CLIA '88.[69] Individual workload limits apply to slides screened per hour and in any given 24-hour period. Screening rates must be monitored to ensure compliance with the workload limits established for each individual.

V.D. Screening Process

Screening processes vary among cytologists based upon experience level, personal preference and other factors. However, certain procedures should be followed. The process of screening should always begin with a check of slide identification (name and/or identifying number) against the accompanying accession slip, test request or pertinent lab document. The examiner must consider available patient history provided by the ordering clinician.

The screening process usually begins with a low power scan of the specimen to assess background and overall adequacy. The actual screening process is usually performed with a 10X objective and 10X or 15X eyepieces. Higher magnification is used for more detailed observation of potentially abnormal areas. The slide should be screened in a systematic and thorough process.

The individual screening the slide is responsible for assessment of adequacy in addition to locating and identifying reportable findings. These findings include premalignant or malignant cells, reactive or reparative features, microorganisms and any features that are not consistent with the clinical history. The location of any abnormal cells or reportable findings should be marked in a consistent pattern by all cytotechnologists within the laboratory to facilitate review. When marking slides, care should be taken to avoid obscuring other significant cellular material.

V.E. Recording results and hierarchical review

After examining and marking the slide, the cytotechnologist records his or her findings. All findings must be recorded accurately, legibly and precisely for future reviewers and data entry personnel. Cytotechnologists should be able to discuss the basis of their interpretations as well as demonstrate them at the microscope. All slides demonstrating reactive or reparative cellular changes and those with epithelial cell abnormalities must be referred to a qualified pathologist for final interpretation.

V.F. Variability in Practice

There are variations in cervical cytology analysis. To some extent, these variations are due to patient and client preferences, disease prevalence, laboratory resources, and market penetration of new technologies. Variability also includes differences in laboratory staff training and experiences,[70] application of microscopic criteria, cytologist/support staff organization and availability of state-of-the-art laboratory information systems. Laboratories may use automated screening devices, liquid-based technology and/or conventional preparations. Hierarchical review may include rescreening by a supervisory level cytotechnologist before examination by a pathologist, or primary screening by pathologists and final sign out without a cytotechnologist. Variations in the methods employed to assess competency of newly hired cytotechnologists also exist.

There is also variability in the mechanics of slide screening. There are personal and laboratory preferences for the utensils used to mark reportable findings on a slide. These include manual

dotting using a felt-tip pen or liquid ink on a sharp-tipped applicator, utilizing a manual device that attaches as a microscope objective to place an ink ring around cells of interest, utilizing a device that attaches to the 10X objective and is triggered electronically to place an ink dot next to the cells of interest and utilizing a device that electronically records the coordinates of areas of interest noted by the cytotechnologist, for subsequent hierarchical review.

For many of these variations of practice, the cytology literature contains little or no data gathered in comprehensive studies to permit conclusive recommendations regarding any one best practice. The College of American Pathologists has collected ASCUS/SIL ratios and other data from laboratories using its Interlaboratory Pap Comparison Program and the Q-Probes questionnaire, enabling individual laboratories to benchmark themselves against distributions of performance.[71, 72, 73] Many articles or textbook chapters present statements of opinion or descriptions of purported optimal practice. However, these practices may not be based on statistically significant data. There have been a number of individual reports that describe particular testing environments in detail,[74] and one has displayed screening speed and accuracy data in a large laboratory setting.[75] The College of Medical Laboratory Technologists of Ontario has recently completed a document, "Practice Guideline: Workload Guideline for Cytotechnologists," which will have regulatory authority in Ontario, Canada.[76] However, comprehensive and definitive laboratory trials assessing differing slide review speeds, hierarchical review algorithms and patterns of task execution as possible influences on result accuracy have not yet been performed.

VI. Cervical Cytology Reporting

VI.A. Specimen Description/Clinical Information

The final report should include the information provided on the requisition such as the menstrual status and any previous history that places the patient in the high-risk category (e.g. history of abnormal cytology results or biopsies, history of cancer). History from the clinician regarding contraception, exposure to exogenous hormones, chemotherapy, or radiation therapy is also important for proper interpretation of cytologic findings. Incorporating the given clinical history in the report assists the clinician in correlating cytologic and clinical findings.

VI.B. Reporting of Specimen Adequacy and Cytologic Findings

The Bethesda System (TBS) of cervical cytology reporting, developed at the 1988 NCI workshop and updated in 1991, was formulated as a means to help standardize the communication of cervical cytology diagnoses.[77] TBS reports have three basic components: a descriptive interpretation, a statement of specimen adequacy, and, optionally, a general categorization of the interpretation. In addition, laboratory and hospital accreditation groups (CAP,[78] JCAHO[79]) have also imposed general requirements on all laboratory reports. Federal regulations require the use of narrative descriptive nomenclature, but do not specify the use of any particular reporting system. Most laboratories use TBS or a modification of it for reporting cervical cytology results.[80]

The adequacy statement of TBS was developed as a standardized means of communicating the quality of the specimen. The statements "satisfactory for evaluation", "satisfactory but limited by" and "unsatisfactory" indicate whether or not the specimen is likely to be sufficient to fulfill the test's screening purpose. The number of cells, cell composition and ability to clearly visualize the cells are factors that are considered in assessing adequacy and are specified in TBS. The statement "satisfactory but limited by…." (with the reason specified) indicates to the clinician that the interpretation is qualified because of the limiting factor. The adequacy statement also provides important feedback to clinicians regarding specimen collection and preparation techniques, contributing to continuous quality improvement. The adequacy statement may also indicate to the clinician the need to consider the option for early repeat testing.[81, 82, 83]

The Bethesda System allows for an optional interpretative statement labeled "general category". The three general categories are within normal limits (WNL), benign cellular changes (BCC), and epithelial cell abnormality (ECA). These designations were developed for report triage and statistical monitoring. For all cases not interpreted as WNL, the report must include a descriptive interpretation that characterizes the cellular changes or abnormality. The category BCC includes specific infections and changes associated with inflammation, repair, contraceptive use, radiation, and atrophy. Some cervical/vaginal cytology specimens with reactive cellular changes will vary in interpretation when examined by multiple individuals.[84] Studies of women with reactive cervical/vaginal cytology on follow up biopsy have found some intraepithelial lesions.[85]

The category ECA includes changes in squamous and glandular cells ranging from atypia to invasive carcinoma. The nonepithelial malignancies encountered less commonly may also be classified here. For squamous lesions, TBS terminology includes atypical squamous cells of undetermined significance (ASCUS), low grade intraepithelial lesion (LGSIL or LSIL), high grade intraepithelial lesion (HGSIL or HSIL) and squamous cell carcinoma. Some laboratories also incorporate other terminologies of dysplasia and/or cervical intraepithelial neoplasia (CIN) into their reports. For glandular lesions, TBS terminology includes atypical glandular cells of undetermined significance (AGUS) and adenocarcinoma. AGUS includes abnormalities of endocervical and endometrial cells. Some laboratories specify whether the cell of origin is most likely endocervical, endometrial or extra uterine. Endocervical adenocarcinoma in situ is reported separately by some laboratories, but in TBS is included in the AGUS category.

VI.C. Variability in Practice

Laboratories may include recommendations as part of the cervical cytology report. These may include a suggestion to the clinician for repeat cytology after a certain time interval or after treatment, or for tissue studies to further evaluate epithelial cell abnormalities. Because medical literature in this area does not indicate a consensus approach, this is one of the most variable elements of cervical cytology reporting among laboratories. Clinical professional organizations have issued Consensus Guidelines for the follow-up of abnormal Pap smear reports. Listing these consensus Guideline references on abnormal Pap reports is useful for alerting the clinician to the Guidelines (JAMA Interim Guideline,[81] ACOG Technical [82] ASCCP Guidelines.[86, 87, 88]) Furthermore, a CAP Q-Probe study of 348 laboratories showed that placing a specific follow-up

recommendation on the Pap report significantly increased the likelihood of the recommended follow-up being carried out.[89] Of course, implicit in any recommendation by a clinical laboratory to a clinician is that the clinician consider all known clinical circumstances and apply appropriate standards of care to their decision to follow, reject, or modify the lab's recommendation for any individual patient.

Reporting of ASCUS and AGUS and recommended patient follow-up, for example, is variable in a number of respects. Numerous studies on follow-up of ASCUS or AGUS have been reported or are in progress. These not only indicate variability of microscopic criteria in use among laboratories, but they also recently have added the element of cost-effectiveness to clinical decision making and the value of alternative follow-up approaches.[23, 90, 91,]

Some laboratories have chosen to include an educational explanatory note, sometimes also referred to as a "disclaimer", on all cervical cytology reports. These notes may have several possible components. They generally note that the Pap smear is a screening procedure with the potential for false negative and false positive results. These statements serve an educational function for the clinician and are designed to encourage a dialogue between patient and clinician. They are not directed to, nor intended to be directly relied upon, by the patient. The dialogue should include the limitations of cervical cytology, an explanation of the various enhanced testing options, repeat testing intervals and any additional follow up that may be necessary.

Recently, articles and exchanges of correspondence in medical journals have addressed the content of such explanatory notes and whether or not laboratories are legally obliged to provide them; consensus is lacking among experts as to recommended practice(s).[92, 93, 94, 95, 96, 97] Until further consensus is reached within the profession, the use of such explanatory notes remains at the discretion of the laboratory director. At present, there is general consensus that the clinician is in the optimal position to assess and apply follow up protocols for individual patients, and should never place sole or unquestioned reliance on the laboratory's suggestions or recommendations.

VII. Quality Control and Quality Assurance Practices

Quality Control is defined as a system for verifying and maintaining a desired level of quality in an individual test or process. Quality control activities span the testing process from the moment of specimen collection until the time the physician receives the report. Quality Assurance (QA) is defined by the College of American Pathologists as systematic monitoring of quality control results and quality practice parameters to assure that all systems are functioning in a manner appropriate to excellence in health care delivery.[98] Quality assurance is a coordinated system designed to detect, control and prevent the occurrence of errors and, ultimately, to further a clinician's ability to appropriately care for his or her patient. A number of quality control/quality assurance measures for cytopathology have been specified by the Clinical Laboratory Improvement Amendments of 1988.[99] All quality assurance processes must be described and documented in a quality assurance program in the laboratory.

VII.A. Pre-analytical Quality Control

Each laboratory must perform and maintain records of routine quality control relating to specimen receipt, preparation and staining. Most of these activities are required by lab accreditation agencies and include such things as review of stain quality and maintenance records, microscope and instrument maintenance, as well as instrument calibration records.[100]

VII.B. Screening and Reporting of Gynecologic Specimens

Federal regulations require that the individual examining a gynecologic cytology specimen be a qualified cytotechnologist or pathologist in a certified laboratory.[67] These individuals may examine up to 100 slides per 24 hours (average 12.5 slides/ hour) and in not less than eight hours. This number is not a performance target but a maximum allowed by law. Pathologists are limited by this ceiling when they perform primary screening. Each laboratory must establish individual workload limits for each cytotechnologist. [69] These limits must be reviewed every six months by the Technical Supervisor of the lab and re-assessed using lab defined performance standards. The record of slides reviewed by the primary screening cytotechnologist or pathologist must be documented and retrievable for inspectors during the retention period prescribed by CLIA ’88 or applicable state law. Cytotechnologists and pathologists must also maintain work logs for any primary screening site (in cases of multiple site employment), again, for the applicable retention period. As discussed in section VI, all specimens must be reported using descriptive nomenclature; use of a numerical reporting system alone is unacceptable. [101]

VII.C. Review of Abnormal Gynecologic Cases

A cervical cytology specimen initially evaluated by a cytotechnologist as reactive, reparative, atypical, premalignant, or malignant must be referred to a pathologist for final interpretation and final report. Discordance between pathologist and cytotechnologist interpretation is often used as a basis for identifying areas for continuing education. Peer review is often included in a quality assurance program. Multiple people may review difficult or interesting cases for educational and interpretive purposes. Seeking the opinion of an outside consultant may be considered for unusually difficult cases with significant clinical implications. Documentation of all reviews is essential for quality assurance monitoring.

VII.D. Rescreening of Negative Cases

CLIA ’88 regulations specify that at least 10% of samples interpreted as negative by each cytotechnologist be re-screened by a pathologist or a qualified supervisory cytotechnologist prior to reporting. Specimens from women considered to be at increased risk for cervical cancer must be included in the review process. Risk status may be determined by review of patient history provided by the clinician on the current requisition. The laboratory must have a clearly defined policy of its definition of high risk as well as its method for random selection of cases.[102] Automated re-screening of negative cases has different requirements. (See VIII.D.)

VII.E. Cytology-Histology Correlation and Clinical Follow Up

The laboratory must compare all pre-malignant and malignant gynecological cytology reports with subsequent histopathology, if available, and determine the causes of any discrepancy. Cyto-histologic correlation can be a helpful educational tool used to refine methods of evaluation for both cytology and biopsy specimens. The correlation process should be documented in the laboratory quality assurance program. Cyto-histologic correlation may be performed prospectively at the time of histologic review with integration of the correlation into the biopsy report. Negative biopsy specimens in the context of recognized SIL or cancer by cytology often indicates a surgical sampling discrepancy.[103] Comments regarding such cyto-histologic discordance in the surgical pathology report may be helpful in directing further patient management. Correlation may also be performed retrospectively. The laboratory must have a clearly defined policy regarding the methods used for cyto-histologic correlation.

If histologic material is not available, the laboratory may attempt to obtain follow-up material or information on patients. This is frequently achieved by sending a letter to the ordering physician requesting follow up information.

VII.F. Retrospective Reviews

Federal regulations stipulate that all negative cervical cytology obtained within the last five years must be reviewed when a new high-grade squamous intraepithelial lesion or carcinoma is detected by cytology. This review includes all available negative smears in the laboratory (either on site or in storage.) If significant discrepancies are detected that would affect *current* patient care, the clinician must be notified and an amended report issued.[104] It is up to the technical supervisor of the laboratory to define significant discrepancy in the laboratory standard operating procedure manual. Retrospective reviews rarely detect abnormalities that affect current patient care.[72] Therefore, amended reports are almost never indicated. However, documentation of the fact that the review occurred should be made separately in internal quality assurance records. Where the review does not result in the issuance of a corrective report, CLIA does not require that specific interpretive discrepancies be documented. Retrospective reviews are subject to the biasing effect of knowledge of outcome, and this fact should be kept in mind during any such review. The main benefit derived from 5- year retrospective review is education of the laboratory staff.

Bias due to knowledge of clinical outcome, context of slide examination and hindsight all plague retrospective reviews. Every reasonable effort should be made to minimize bias when reviewing cases/slides for laboratory or individual performance evaluation. There are a number of methods to attempt this including:

- Review by multiple individuals,
- Review without knowledge of clinical outcome,
- Review of the index case embedded in a slide sequence containing a range of normal and abnormal cases.[105]

VII.G. Measures of Screening Performance

Cervical cytology is a highly successful screening test. Cervical cytology is limited (as are *all* screening tests) by both false positive (FP) and false negative (FN) results. A false positive is

defined as a "positive" test result for a woman who does not have a cervical abnormality. "Positive" results are variably defined in the medical literature; however, squamous or glandular intraepithelial lesions or cancer are the most reproducible benchmarks defining a positive result. There are multiple reasons for false positive cytology. For example, LSIL may be present at the time of the screening Pap test and the lesion may have regressed prior to biopsy, or a small lesion may not have been sampled with colposcopically directed biopsies or ECC.[103] False positives are likely to occur at some level because of the difficult, subjective, interpretive character of cytologic evaluation, and due to pressures to minimize false negative results.

A false negative is defined in this document as a negative cervical cytology test result in a woman with a cervical squamous or glandular intraepithelial lesion or cancer. The false negative rate for high grade intraepithelial lesions likely to progress to cancer and for invasive cancer itself is of greatest concern to all parties involved in the screening process. False negative results may be a consequence of (a.) Patient sampling by the clinician or (b.) Laboratory screening or interpretation. Sampling false-negatives occur when abnormal cells from the lesion are not collected or are not transferred to the slide. A laboratory screening or interpretive false negative is one in which abnormal cells are present on the slide, but are not identified by screening or are misinterpreted after being noticed during screening.

The false negative rate is the sum of lesions missed in sampling plus the false negative proportion (FNP.) The FNP is the measure of the laboratory component of false negative results and is defined as the number of false negative reports divided by the total number of women screened who have a cervical abnormality (False Negative Proportion = False Negative reports/True Positive reports + False Negative reports).[106, 107, 108]

$$FNP = \frac{FN}{TP + FN}$$

The value of determining the FNP for a laboratory is widely acknowledged; however, precise calculation of the FNP requires both 100% re-screening of negative cases and unachievable 100% accuracy. The accuracy of rescreening is the major variable that affects the calculation. In everyday practice, the FNP may be <u>estimated</u> based on rescreening a sample of cases selected at random. The best estimates of true false positive and false negative rates are achieved from large prospective studies in which all slides are independently reviewed and differences of opinion are resolved by an independent panel of cytologists. [109] Based upon data collected in the medical literature, it may be extremely difficult to reduce the FNP below 5 to 10%. [110] The false negative proportion calculated for a laboratory represents an estimate of the staff's average screening sensitivity. If sampling false negatives are added to the laboratory FNP, the overall false negative rate of cervical cytology may approach 20% or higher.[111]

The threshold of abnormality used to define FN and TP must be consistent and every effort to reduce bias should be undertaken. For laboratory and individual performance, a false negative threshold of either ASCUS or LSIL may be used. An LSIL threshold is preferred because the degree of reproducibility of an ASCUS/AGUS interpretation is low.[84, 112]

CLIA ’88 mandates that a laboratory must evaluate individual performance in comparison to overall laboratory performance. Regulations do not mandate any specific method of evaluation. Most frequently used measures include: random rescreening, targeted rescreening of specific patient groups, seeding abnormal cases into the screening and rescreening pools, and retrospective rescreening of negative cervical cytology specimens from patients with a current high grade abnormality. Retrospective rescreening evaluates past rather than current performance and is therefore difficult to statistically standardize for comparison of screening performance. Statistical measures may include comparison of an individual’s FNP to that of the overall laboratory. Regardless of the method used the laboratory should establish performance expectations, document performance in comparison to these expectations, and have a program for corrective action when individuals do not meet the laboratory’s specific requirements.

VII.H. Proficiency Testing and Continuing Medical Education

Proficiency testing has been mandated under CLIA ‘88 for individuals examining gynecologic specimens.[113] To date, a national system has not been devised. However, a number of state and private programs provide proficiency evaluation. Examples include:

1. State of Maryland Gynecologic Cytopathology Proficiency Program (HCFA approved)
2. New York State Cytopathology Proficiency Testing Program
3. CAP Interlaboratory Comparison Program in Cervicovaginal Cytopathology
4. CytoQuest ® Glass Slide Program from Midwest Institute for Medical Education (MIME)
5. CheckSample ®, CheckPath ® and STAR® Programs from the American Society of Clinical Pathologists

Liquid-based cervical cytology specimens should be included in proficiency testing programs for laboratories that use this methodology.

Ongoing education is a requirement for proficiency in cytology. This requirement can be fulfilled by participation in proficiency testing, intradepartmental slide review sessions, attending workshops and symposia, teaching cytotechnology students, pathology residents and fellows, independent study, and community outreach programs. To maintain professional licensure, some states and professional societies have varied requirements for continuing medical education.

VII.I. Variability in Practice

The total percentage of negative cases rescreened, and selection method will vary among laboratories. Some labs may randomly select 10% of the negative smears from a combination of both high risk and non-high risk patients. Other labs may select 10% of non-high risk cases in addition to some or all high-risk cases for re-screening. Since accuracy of rescreening has a major impact on a laboratory’s estimate of its screening false-negative rate, efforts to optimize the accuracy of rescreening are as important as efforts to optimize the accuracy of primary screening. This should be taken into account in a laboratory’s assignment of rescreening duties. Laboratories using automated screening devices at a minimum must follow the manufacturers’ directions that have been approved by the FDA and deemed compliant with CLIA regulations according to HCFA.

VIII. Data Management and Laboratory Information Systems

Manual methods as well as computerized systems exist for management of laboratory data. Manual methods may include logs and card files organized by date, patient name, specimen number or interpretation. Computerized systems, most often referred to as laboratory information systems (LIS) may stand alone, be part of an integrated anatomic pathology system, part of a multispecialty laboratory system, or integrated with a larger hospital or corporate information system. This section of the Guideline describes data management components needed to generate the information used by the laboratory, clinicians and other healthcare organizations.

VIII.A. Record Storage and Retrieval

The laboratory must have the ability to record and retrieve specimen information and patient reports for the periods specified by regulatory agencies.[61, 114] The system, whether manual or automated, should allow access to all cytology reports and all available and related surgical pathology reports to facilitate cytologic/histologic correlation. Older data may be electronically archived or records may be stored offsite as long as retrieval does not hinder patient care or delay regulatory inspections. The ability of a system to correlate or merge records when there is an alteration in patient identifiers (such as name, hospital record number or other identifiers) without altering the data in the original records is also desirable. The use of unique identifiers, such as the patient's hospital record number, allows for more accurate matching.

VIII.B. Accessioning and Work Flow

The laboratory must assign a unique accession number for each individual case. All patient demographic data required by regulatory agencies should be entered at accessioning. The unique accession number facilitates the tracking of a case through all stages of handling in the cytology laboratory from pre-analytic (accessioning and specimen preparation,) and analytic (screening and interpretation,) through post-analytic processing (reporting, and quality assurance follow up.) [115] Labels for paperwork and slides may be handwritten, purchased, printed with a stand-alone printer or generated by the LIS as part of accessioning. Bar coded labels can increase the efficiency and accuracy of this process.

VIII.C. Security

All laboratory records are confidential. Access should be limited to authorized individuals. Locked cabinets for paper records and security codes for electronic systems are recommended. Limiting access may deter corruption of computer software or inadvertent change or release of results by unauthorized individuals. Electronic signatures are preferable for reports that are stored in electronic format. A procedure should be in place to assure that the electronic signature identifies the person who is responsible for the case and indicates that they approve of the content of the report. This procedure should prohibit interpretations that require pathologist review from being released by any other individual prior to the pathologist's authorization.

VIII.D. Terminology

Standardized terminology (The Bethesda System or other comparable system) used in the LIS should be stored in the computer database and accessed by use of mnemonics or assigned codes. Free-text capabilities are necessary for rare or unusual interpretations or for comments and/or recommendations that are not routine. Manual reporting should be standardized to allow retrieval of data based upon interpretation.

VIII.E. Data Transfer

Transfer of clinical information and interpretive data to the report must be precise. This may occur via a manual written report, by manual entry into the LIS, or by use of optical mark readers that are interfaced with the LIS. The accuracy of this information must be monitored through the laboratory's Quality Assurance Program.[116] In addition to storing patient information and reports, laboratory information systems (LIS) may be used to generate billing statements or to transfer data to billing systems, clinician offices, hospital computer systems, Medicare, and other third party payers. Linkage of reports to interpretation and procedure codes [International Classification of Disease (ICD-9)], hospital procedure and billing codes [HCFA Common Procedure Coding System (HCPCS)] and Current Procedural Terminology (CPT) codes may be required for billing purposes. Linkage of reports to SNOMED (Systematized Nomenclature of Medicine) is desirable for statistical reporting.

VIII.F. Quality Assurance

Laboratory data must be retrievable for quality assurance purposes and to generate statistical reports required by regulatory agencies and accrediting organizations within the retention period prescribed by CLIA '88 (2 years) or applicable state regulations. The system should provide the breakdown of the interpretive categories reported by each individual. This individual statistical data must be available for comparison with the laboratory average.[117]

It is desirable for the LIS to facilitate the selection of cases initially screened as negative for random and directed rescreening. The laboratory must not allow release of results until the rescreen examination is complete. Results of rescreening should be available for calculation of false negative proportions or other measures of performance within the retention period prescribed by CLIA '88 (2 years) or applicable state regulations. Cytologic/histologic correlation information needs to be available for review (again within the retention period prescribed by applicable regulations.) The data management system must allow the laboratory to follow-up premalignant and malignant lesions and monitor unsatisfactory rates by clinician.

VIII.G. Variability of Practice

Differences between manual and electronic data management systems are discussed throughout this section and encompass most practice settings.

IX. Enhancements to Conventional Cervical Cytology Testing

New technologies are available or are in development that are designed to increase the sensitivity of cervical cytology screening and may enhance other aspects of laboratory performance. Each technological device may have strengths and weaknesses.

IX.A. Liquid Based Methods

Liquid-based processing (LBP) methods are designed to improve cervical cytology specimen adequacy by improved cell harvest and application of the cell sample to the slide, decreased obscuring factors and decreased air drying artifact. A LBP technique can be achieved by a number of methods. Currently, the USA's Food and Drug Administration (FDA) has approved one filter-transfer method and one density-gradient method.[118, 119] Studies from different practice environments may show variable results pertaining to improved adequacy and sensitivity, probably due to differences in pre-analytic and analytic factors (e.g. the patient population served, sample taker proficiency, laboratory conditions, the experience and proficiency of laboratory personnel.) The decision of whether or not to implement LBP methods and which methods to employ should be based on an assessment of the likelihood of improved performance in the particular practice setting.

IX.A.1. Pre-analytic (Sampling and Processing) Considerations

Consideration should be given to using the optimum sampling device for a particular technology. Both current LBP methods have been approved for use with the "broom-type" devices. The plastic spatula and the endocervical brush have also been approved for use with the filter-transfer method. The use of other sampling devices or combinations that are valid for conventional smears should not be presumed to be optimal for LBP in the absence of evidence.

To obtain intended performance, the manufacturer's recommended processing procedures must be followed. Results are dependent on careful technique.

IX.A.2. Analytic (Screening and Review) Considerations

Only personnel who are trained and certified in these methods should perform the screening and review of the slides. This training may be provided by the manufacturer or accomplished in the laboratory by the manufacturer's certified personnel.

IX.B. Automated Screening Devices

Automated screening devices rely on computer analysis of digitized images of cells to triage cervical cytology slides for subsequent identification of premalignant and malignant changes. One device has received FDA approval for use both in a quality control rescreening mode, and as a primary screening device.[120] The potential benefits from these types of automated screening instruments include reduction of false-negative rates, increased sensitivity, and increased throughput for the laboratory.[109]

IX.C. Microscope Process control Systems

Microscope process control systems are designed to assist with quality control and quality assurance. By mechanizing and automating certain steps of the screening process, the entire slide or predesignated portion of the slide is presented to the microscopist. The percent overlap during screening, the direction of screening (vertical or horizontal), the mode of screening (continuous or field by field) and the speed of screening can be automatically set to default values or can be adjusted to fit the individual examining the slide. These process control systems are equipped with electronic marking capability that expedites the relocation of cells for review. In addition, some have a mechanical pen that marks the areas of interest on the slide. The cytologic interpretation for each mark can be keyed in by the cytotechnologists for evaluation by the cytopathologists, allowing the pathologists to compare their interpretations with that of the cytotechnologists. The movement and coverage of the slide, the time spent on the stage, the number and location of marks, the interpretation of the cytotechnologist relative to each mark, and the final interpretation, are all available in real time when using a process control system. Thus, statistical data is generated that can be used for quality assurance and quality control.

IX.D. Molecular and Immunologic Techniques

Adjunct testing for low and high-risk HPV subtypes is currently available. HPV testing represents an option in the triage or management of women with a cervical cytology interpretation of ASCUS.[86, 121]

IX.E. Variability in practice

The decision to implement technologic enhancements to cervical cytology screening is affected by the following:

- Perceptions of current laboratory performance and screening accuracy by laboratory management, pathologists, cytotechnologists, clinicians and patients
- Effectiveness of the technology to improve performance and accuracy
- Technical limitations (e.g., slide preparation devices may not be compatible with screening devices)
- Cost
- Availability for various sectors of the population

Studies addressing decision analysis and cost-effectiveness of technological enhancements to cervical cytology screening have been published.[12, 122, 123, 124] Large scale randomized and blinded clinical studies that compare the new technologies to conventional cervical cytology and to one another would be useful. Rigorous evaluation of these studies will facilitate evidence-based decision-making pertaining to these enhancements.

X. Archiving and Interlaboratory Slide Review

X.A. Slide Storage and Retrieval

Cytology laboratories must retain all cervical slide preparations, regardless of diagnosis, for five years from the date of microscopic examination, or for longer if state regulations require.[114] Slides may be stored on-site in the laboratory or on institutional premises, or may be stored off-site. Whether stored on-site or off-site, slides must be retrievable within a reasonable amount of time if retrospective review is necessary (see VII.F.) or as requested for external inspection procedures (see XIV.E). Slide breakage and slide loss may occur on rare occasions. When breakage is discovered, there should be appropriate documentation of the incident and repair of the slide if possible.

X.B. Records Storage and Retrieval

As is the case with storage and retrieval of slides, records may be stored on-site in the laboratory or on institutional premises, or may be stored off-site. Whether stored on-site or off-site, records must be retrievable within a reasonable amount of time if retrospective review is necessary (see VII.F.) or as requested for external inspection procedures. Again, required retention periods under CLIA ’88 or applicable state regulations, vary depending upon the type of record (see section X.C.).

If reports are stored in a computerized information system with appropriate backup, as microfilm, or as microfiche, laboratories are not required to retain paper copies of reports. Such stored report records must contain the same information (“exact copy”) that is sent to the authorized individual who orders or utilizes the test report. However, it is not required that an "exact copy" be an exact duplicate of the report. Exact copies must also contain the signatures (electronic or manual) when required.[125]

X.C. Retention Requirements

While State, local or professional requirements may require longer retention timeframes, current federal regulations mandate the following retention periods for materials related to cervical cytology specimens: [43, 114, 126]

- Test requisitions must be retained for 2 years from date received
- Test reports must retained for 10 years from date of the report
- Logs and accession records for cervical cytology specimens must be retained for 2 years from date of receipt.
- Quality control records for cervical cytology specimens must be retained for 2 years from the date that they were created/generated.
- Documents pertaining to discontinued procedures for cervical cytology specimens must be retained for 2 years from the date that they were discontinued.
- Maintenance records for instruments used in processing and analyzing cervical cytology samples must be retained for 2 years after the instrument(s) has been out of use.
- All cervical cytology slides, regardless of diagnosis, must be retained for 5 years from date of examination.

X.D. Loaning of Slides for Proficiency Testing Programs and Interlaboratory Slide Review

Slides that are less than five years old may be loaned to proficiency testing programs in lieu of maintaining them for this time period. The laboratory must receive acknowledgment of the receipt of slides by the proficiency-testing program and maintain documentation of the loan of such slides thereby allowing retrieval the slide(s) if needed. Documentation of slides less than 5 years old that are loaned or referred for purposes other than proficiency testing (such as for interlaboratory slide comparisons, consultation, or educational purposes) also must be maintained.[114]

X.E. Discarding Slides and Records

Slides and records that are outside retention and retrieval requirements may be discarded. When discarding such materials, patient confidentiality must be insured. The disposal process must result in the inability to identify the patient. If outdated/expired materials are retained for educational or research purposes, then patient identifiers should be removed.

X.F. Requirements for Cervical Cytology Materials Received from or sent to Secondary Laboratories (Reference or Referral Laboratories)

The laboratory in which the slides were actually examined for final interpretation must store the slides.[61, 114] A reference or referral (secondary) laboratory is responsible for storing slides interpreted in that laboratory for the 5-year retention period. For retrospective review purposes, the reference or referral laboratory must review previous cases stored in the laboratory's files, but is not required to request previous slides from another laboratory for this purpose. The report must clearly state which laboratory performed the interpretation.[125]

X.G. Variability in Practice

- Slide retention requirements for state and federal regulations and professional accreditation organizations may vary. Both CLIA ’88 and state regulations should be consulted.
- Academic and research goals may merit longer slide storage by individual laboratories.
- Restrictive slide storage and access policies may be necessary on the basis of federal regulations mandating slide storage and custody.
- The systems by which laboratories retain, store and retrieve slides and records vary. For example, laboratories may store these materials in accession number order, by patient name, by date received or reported, by interpretive categories or by other means.

XI. Laboratory Cost Accounting and Financial Management

XI.A. Methodology for Cost Accounting

The need for comprehensive cost-accounting data reflecting the true direct and indirect costs of laboratory cervical cytology testing services was recently highlighted by an "Inherent Reasonableness Review of Payment Rates for Pap Smears" conducted in early 1999 by the Health Care Financing Administration.[127] HCFA acknowledged that the $7.15 Medicare Pap smear reimbursement rate in place in early 1999 was based on a survey of a single laboratory in 1984, with no intervening cost adjustments.[128] In responding to HCFA's request for valid cost data, it became apparent that many laboratories did not know how to correctly determine their total costs for Pap testing. Full cost accounting must include indirect as well as direct costs of testing in order to avoid underestimates, which represent only partial testing costs. Reimbursement or pricing policies based on such incomplete cost estimates may prove inadequate to enable continuation of quality testing in the future.

Several descriptions of cervical cytology cost accounting have been published or presented in public forums. Spires recently published a detailed study of cost allocation per case.[129] Cost allocation categories broadly included: Supplies, Equipment, Depreciation, Maintenance, Specimen/Report Transportation, Non-MD Personnel Expenses, QA, Physician expenses, Laboratory Information Systems, Overhead, Other, and Billing/Bad Debt Expenses. Another relatively detailed Pap cost accounting scheme was presented at the April 1999 ASC Cytopathology Review in Columbus, Ohio.[130] Broad cost categories included: 1) Accessioning, history match, staining, cover-slipping, and clean-up; 2) Cytotechnologist review; 3) Result data entry; 4) Pathologist review; 5) Facilities; 6) Billing and collection costs; 7) Malpractice costs; 8) Logistics, and 9) Administrative costs such as sales, general management, finance and other support functions. Omission of any cost category will significantly underestimate the true cost of cervical cytology. Important QA functions such as training and continuing medical education are unfortunately and inappropriately omitted from cost analyses.

Each laboratory should establish its own cost for providing cervical cytology services, recognizing that costs are dynamic. Ideally, cervical cytology costs should be monitored and adjusted as needed. New technologies, availability of cytology professionals, and expectations for performance of cervical cytology screening will continue to affect cost.

Recent testimony, by the American Society for Clinical Pathologists on March 16, 1999 before the US House of Representatives Commerce Subcommittee on Health and the Environment, estimated the actual laboratory cost of testing a conventional cervical cytology smear in the range of $13 to $17. This included cytotechnologist salaries as well as overhead costs, CLIA-mandated quality control, laboratory supplies, and supplies given to health care providers who obtain the smear.[131] This range of cost reflected a consensus of a broad coalition of professional organizations, including the ASC, which was presented to HCFA during a meeting held in Baltimore, Maryland in February 1999.[128, 132, 133] Professional organization efforts to improve cervical cytology reimbursement to more realistic levels culminated in passage of legislation in late 1999 with Medicare reimbursement for the conventional Pap smear increasing to $14.60.[134]

XI.B. Cost Accounting Issues

To date, only limited cost accounting data in relation to the new cytology technologies are available. This is not surprising, since these products have only been on the market for several years, with variable market penetration. Several recent technology assessment reviews have published estimated costs associated with the new cytology enhancements.[12, 135, 136] There has been considerable debate over the accuracy of the estimates. Accurate cost accounting of the new cytology technologies is needed, since each of the currently available devices increases the cost of testing. Reasonable reimbursement by third-party payers is needed if these technologies are to become available to large groups of patients, especially the underserved.

To the extent that cytopathology laboratory directors understand and communicate testing cost data and rely on accepted accounting principles, the chronic problem of below-cost reimbursement for cervical cytology services may gradually be alleviated; the incentive to offer a wide variety of quality enhancements will increase commensurately. Many laboratories have incomplete knowledge of their actual direct and indirect costs for offering this service, which has contributed to unrealistically low pricing. Offering cervical cytology services as a below cost "loss leader" in order to procure other profitable business may represent an ethically questionable practice and from a legal perspective may be considered inducement. It has the effect of discouraging expenditures needed for optimal cytology practice and hinders long-term development of the specialty. Below-cost reimbursement practices have delayed the introduction of more expensive new technology enhancements. Efforts to achieve adequate reimbursement represent a major quality-related issue for gynecologic cytology in the twenty first century.

XII. The Role of the Cervical Cytology Practice Guideline

XII.A. Intended Use

Guidelines must reflect consensus and have broad application in professional practice.[137] As indicated in the Introduction, this Guideline focuses on the practical aspects of cervical cytology screening. Its purpose is educational and proscriptive. It is a compilation of current practices, laboratory accreditation standards from professional organizations, and regulatory requirements in the United States. It reflects the spectrum of technical and analytical procedures that are available and commonly practiced in contemporary cytopathology laboratories. The Cervical Cytology Practice Guideline provides guidance for cytologists, clinicians, patients and others involved in cervical cytology screening.

XII.B. The Role of Variability in Practice

Variability in analytical and technical methodologies does not imply an undesirable lack of standardization. Differences may reflect practice variations that are dependent upon individual laboratory resources, client needs, and patient population. A cytology laboratory may legitimately elect to use preparation methods, analytical processes, interpretive terminology and/or reporting comments that differ from those described in this document or those used in most laboratories. These variations in practice, if conducted in accordance with regulatory and professional oversight, and documented in laboratory procedures, should be viewed as reasonable and customary.

XIII. Mechanism of Scheduled Review

The American Society of Cytopathology (ASC) Cytopathology Practice committee completed this Guideline in 2000. The Guideline was reviewed by the ASC Executive Board (EB) and the ASC committee chairs and was presented to the general ASC membership at the 48th Annual Meeting in November 2000.

This document will be reviewed and updated periodically. Once endorsed, updated versions will be forwarded to the American Medical Association and to the National Guideline Clearinghouse.

XIV. APPENDIX

XIV.A. Creating a Guideline Document

Since the late 1980's "guidelines" have attained growing importance. Guidelines draw from relevant literature, educational and workshop curricula and important elements of daily clinical practice. They are unique and fill important gaps in the literature.

Processes by which Guidelines are created have received systematic study and description.[1]

Five general attributes to guide the development and evaluation of practice parameters/guidelines have been recommended. These are:

1) Practice parameters/Guidelines should be developed by or in conjunction with physician organizations,
2) Practice parameters/Guidelines should explicitly describe the methodology and process used in their development,
3) Practice parameters/Guidelines should assist practitioner and patient decisions about appropriate health care for specific clinical circumstances,
4) Practice parameters/Guidelines should be based on current professional knowledge and reviewed and revised at regular intervals, and
5) Practice parameters/Guidelines should be widely disseminated.

XIV.B. 25 Standards for a Practice Guideline

The format and content of this Guideline are based in large measure on the principles and procedures suggested by Shaneyfelt et al.[138] Modifications to the 25 standards appear in italics.

Development and Format

1. Purpose of guideline is specified.
2. Rationale and importance of the guideline are explained.
3. The participants in the guideline development process and their areas of expertise are specified.
4. Targeted health problem or technology is clearly defined.
5. Targeted patient population is specified.
6. Intended audience or users of the guideline are specified.
7. The principal preventive, interpretive, or therapeutic options available to clinicians, patients *and cytologists* are specified.
8. The health outcomes are specified.
9. The method by which the guideline underwent external review is specified.
10. An expiration date or date of scheduled review is specified.

Evidence Identification and Summary

11. Method of identifying scientific evidence is specified.
12. Time period from which evidence is reviewed is specified.

13. The evidence used is identified by citation and referenced.
14. Method of data extraction is specified.
15. Method for grading or classifying the scientific evidence is specified.
16. Formal methods of combining evidence or expert opinion are used and described.
17. Benefits and harms of specific health practices are specified.
18. Benefits and harms are quantified.
19. The effect on health care costs from specific health *and laboratory* practices are specified.
20. Costs are quantified.

Formulation of Recommendations

21. The role of value judgments used by the guideline developers in making recommendations is discussed.
22. The role of patient *and cytologist* preferences is discussed.
23. Recommendations are specific and apply to the stated goals of the guideline.
24. Recommendations are graded according to the strength of the evidence.
25. Flexibility in the recommendations is specified.

XIV.C. PROCEDURES USED IN THE CREATION OF THE ASC CERVICAL CYTOLOGY PRACTICE GUIDELINE

In 1997 the ASC President charged two committees, the Cytopathology Practice Committee and the Quality Assurance Committee to create a Practice Guideline for cervical cytology. The charge was to address technical, interpretive, information management, quality control and quality assurance, documentation, and medico-legal aspects of cervical cytology. These Committees were composed of experienced and expert cytologists who also enlisted the expertise and knowledge of others to produce a complete and helpful resource. The composition of the group reflected a broad cross section of cytology practices, including cytopathologists or cytotechnologists from small, medium and large hospital laboratories, and representatives from large-scale commercial laboratories.

The first work product was an outline. Next, expanded drafts were created based on the outline. Relevant references were identified and ranked by committee members according to their scientific merit. Content was refined and drafts were circulated for editing. Committee discussions originally centered around the document's content then on purpose and form. Committee members relied at least in part on eight categories of guideline attributes: 1) Validity, 2) Reliability/Reproducibility, 3) Clinical Applicability, 4) Clinical Flexibility, 5) Clarity, 6) Multidisciplinary Process, 7) Scheduled Review, and 8) Documentation.[1]

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XIV.E. NATIONAL REGULATORY REQUIREMENTS AND PROFESSIONAL ORGANIZATION CRITERIA

XIV.E.1. Clinical Laboratory Improvement Amendments of 1988 (CLIA '88)

CLIA ’88 considers cytology as high complexity testing. All mandates that apply to high complexity labs apply to the cytology laboratory. These are listed in the Federal Register.[139] For additional information refer to www.hcfa.gov/medicaid/clia/cliahome.htm.

XIV.E.2. Laboratory Inspection and Accreditation

Laboratories are inspected by the state or by agencies that have received deemed status by the Health Care Financing Administration (HCFA) of the federal government. The two national agencies with deemed status are the Laboratory Accreditation Program (LAP) of the College of American Pathologists (CAP) and the Joint Commission on Accreditation of Hospitals Organization (JCAHO). Organizations with deemed status have inspection requirements that are equivalent to or more stringent than Federal regulations. For additional information refer to www.cap.org/HTML/ftpdirectory/checklistftp.html and www.jcaho.org/trkhco_frm.html.

XIV.E.3. National Committee for Clinical Laboratory Standards (NCCLS)
NCCLS is a non-profit, educational organization that provides a communication forum for the development, promotion and use of national and international standards. NCCLS documents describe laboratory procedures, bench and reference methods and evaluation protocols applicable within all the major laboratory disciplines. It has published "Papanicolaou Technique: Approved Guideline-Second Edition," that describes safety issues and procedures for cervicovaginal specimen collection, preparation, fixation, staining and storage of slides. For additional information refer to www.nccls.org.

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