Cervical Cytology Specimen Adequacy: Patient Management Guidelines and Optimizing Specimen Collection

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■ **Abstract**

Objective. To provide updated management guidelines according to cervical cytology specimen adequacy and techniques to optimize adequacy based on literature review and expert opinion.

Materials and Methods. Selected members of the American Society for Colposcopy and Cervical Pathology committee and invited experts conducted a literature review and discussed appropriate management and areas for future research emphasis.

Results. The guidelines recommend a repeat Pap test in a short interval of 2 to 4 months for most women when the cytology result is unsatisfactory. The preferred follow-up for women with a negative cytology result lacking an endocervical/transformation zone component or showing other quality indicators is a repeat Pap test in 12 months. Indications for an early repeat Pap test in 6 months are provided, and the influence of human papillomavirus testing results on management is discussed. Techniques for optimizing specimen adequacy are provided in detail.

Conclusion. The specimen adequacy management guidelines will help promote uniform and optimal follow-up of patients receiving cervical cytology screening. The topics for future research emphasis will be helpful in promoting studies in needed areas. ■

Key Words: cervical cytology screening, Pap test, specimen adequacy, guidelines, management, Bethesda terminology, unsatisfactory

In 2002, a task force of the American Society for Colposcopy and Cervical Pathology (ASCCP) published a set of guidelines related to Pap test specimen adequacy and patient management following the National Cancer Institute Bethesda 2001 Workshop which updated terminology and reporting of cervical cytology [1, 2]. The 2002 ASCCP guidelines included recommendations on the follow-up of women with either an unsatisfactory Pap test or a Pap test with quality indicators including lack of an endocervical/transformation zone (EC/TZ) component. An unsatisfactory Pap test either shows scant cellularity or has more than 75% of cells obscured and is considered

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Table 1. Rating System for Recommendations

Recommended	Good data to support use when only 1 option is available
Preferred	Option is the best (or one of the best) when there are multiple other options
Acceptable	One of multiple options when there are either data indicating that another approach is superior or when there are no data to favor any single option
Unacceptable	Good data against use
Strength of recommendation	
A	Both strong evidence for efficacy and substantial clinical benefit support recommendation for use
B	Moderate evidence for efficacy—or strong evidence for efficacy but only limited clinical benefit—supports recommendation for use
C	Evidence for efficacy is insufficient to support a recommendation against use, or evidence of efficacy might not outweigh adverse consequences
D	Moderate evidence for lack of efficacy or for adverse outcome supports a recommendation against use
E	Good evidence for lack of efficacy or for adverse outcome supports a recommendation against use
Quality of evidence	
I	Evidence from at least 1 properly randomized, controlled trial
II	Evidence from at least 1 well-designed clinical trial without randomization, from cohort or case-control analytic studies (preferably from more than 1 center), or from multiple time-series studies, or dramatic results from uncontrolled experiments
III	Evidence from opinions of respected authorities based on clinical experience, descriptive studies, or reports of expert committees

unreliable for evaluation of epithelial abnormalities. The absence of an EC/TZ component and partially obscuring factors (50%–75% of the cells obscured) are considered quality indicators but do not make a Pap test unsatisfactory [2]. Since the publication of these guidelines, 2 major changes have influenced cervical cancer screening. First, the majority of Pap tests performed in the United States are now liquid-based preparations (LBPs) instead of conventional smears (CSs). Second, human papillomavirus (HPV) DNA testing for oncogenic/high-risk types is increasingly used in conjunction with cervical cytology as a primary screening test for women aged 30 years and older. Both of these developments have influenced screening methods and frequency that impact the adequacy issues raised in the original ASCCP guidelines. The ASCCP Pathology Committee has revisited and updated these guidelines and has included emphasis on areas for future research and methods to optimize Pap test collection. The guidelines and discussion below are based on published evidence and expert opinion of members of the ASCCP Pathology Committee and other experts in the field. The rating system for recommendations and quality of evidence is identical to previous ASCCP guidelines (Table 1) [3].

RESULTS

Issue 1: What Is the Recommended Follow-up for Women With an Unsatisfactory Pap Test?

Recommendation. The recommended management for most women undergoing cervical cancer screening who have an unsatisfactory Pap test result is a repeat Pap test, generally within a short time interval of 2 to 4 months (AII). This is unchanged from previous recommendations [1]. If the unsatisfactory result is due to obscuring inflammation and a specific infection is identified, consider specific treatment before repeating the Pap test. Additional clinical evaluation is recommended in women with symptoms, abnormal examinations, and in cases where the Pap test is repeatedly unsatisfactory because of obscuring blood, inflammation, or necrosis (BIII) [1]. Examples are women with visible lesions, friable cervix, postcoital or abnormal bleeding, pelvic pain, and abnormal discharge; the additional evaluation may include colposcopy and/or biopsies, as appropriate.

An unsatisfactory Pap test that was not indicated according to the screening protocol in effect (e.g., the patient was not due for her next cervical cancer screen) does not necessarily need to be repeated. Some women may not require continued cytology screening. Women who have had a hysterectomy with removal of the cervix for benign disease do not generally benefit from screening [4–7]. Lower cellularity specimens may be acceptable in women who have undergone hysterectomy for malignancies, chemotherapy, or radiation therapy, as obtaining specimens with higher cellularity may not be possible in these situations [2]. Clinicians and laboratories should exercise judgment in determining whether the specimen is unsatisfactory and whether early repeat cytology is indicated.

Issue 2: What Is the Recommended Follow-up for Women With a Negative (for Intraepithelial Lesion or Malignancy) Pap Test Lacking an EC/TZ Component or Showing Other Quality Indicators (Borderline Cellularity, Partially Obscuring (>50%) Blood or Inflammation)? Should HPV DNA Test Results Influence Patient Management?

Recommendation. The preferred management for most women who lack an EC/TZ component on their Pap and are undergoing routine screening is a repeat Pap test in 12 months; this also applies to other quality indicators (BIII). This recommendation is unchanged from the previous guideline [1]. An early repeat (generally at 6 months) may be beneficial for some women. Indications for considering an early repeat include (1) a previous squamous abnormality (atypical or worse) without 2 subsequent negative Pap tests or a negative HPV test, (2) a previous Pap with unexplained glandular abnormality, (3) a positive high-risk/oncogenic HPV test within 12 months, (4) clinician inability to clearly visualize the cervix or sample the endocervical canal, (5) similar obscuring factor in consecutive Pap tests, and (6) insufficient previous screening.

HPV test data may be available in women with negative Pap tests that lack an EC/TZ component. If the HPV test is negative, it may be prudent to repeat the Pap test in 12 months rather than extend the screening interval to 3 years in women older than 30 years. Negative HPV status generally confers a lower risk for abnormalities; however, only limited data are available at this time indicating the relationship between HPV test status and presence or absence of an EC/TZ component. If the HPV test is positive, it is preferred to repeat the Pap test in 6 months; if cytology is negative at 6 months, repeat HPV testing at 12 months is suggested to check for HPV clearance (CIII).

DISCUSSION

The guidelines in this article primarily address issues related to primary cervical cancer screening with the Pap test, or the combination of the Pap and HPV tests in women aged 30 years and older. Women who are symptomatic or have visible abnormalities or an abnormal examination generally require additional evaluation such as colposcopy, biopsies, or microbiologic evaluation. Due to the known false-negative rate of cervical cytology, a negative result does not exclude an abnormality when a lesion is present. Colposcopy is suggested for women with repeated bloody or inflamed unsatisfactory Pap test results [1].

Issue 1: Unsatisfactory Pap Tests

Unsatisfactory Pap tests include those that are rejected by the laboratory (due to labeling problems, specimen vial leakage, slide breakage, etc.) and those that are completely processed but are unsatisfactory due to insufficient squamous cells or obscuring (>75%) blood, inflammation, or other processes [2]. Most processed unsatisfactory LBP specimens are related to insufficient squamous cells. An unsatisfactory Pap test is considered unreliable for detection of epithelial abnormalities, and several studies have found that women with unsatisfactory results may be at significant risk for disease [8, 9]. Estimating adequate cellularity of specimens with cell clusters, cytolysis, and atrophy is difficult using representative field counts, and laboratories should exercise judgment in reporting such specimens [2].

The numerical criteria for squamous cellularity on Pap tests were developed for women undergoing routine cervical cancer screening and do not apply to vaginal specimens [2]. In posthysterectomy vaginal specimens, laboratories should exercise judgment in evaluating adequacy based on clinical and screening history [2]. Many cytologists accept lower cellularity in vaginal specimens, especially if atrophy is present and the woman is at low risk, but no peer-reviewed data have been published to define the adequacy of vaginal specimens. Until more data are available, laboratories have flexibility in determining methods for cellularity estimation in vaginal specimens.

After hysterectomy, many women may not benefit from additional cytology screening. The American Cancer Society (ACS) guidelines state that screening after total hysterectomy (with removal of the cervix) is not necessary unless the surgery was done as a treatment for cervical cancer or precancer [7]. The American College of Obstetricians and Gynecologists guidelines are similar for women who have undergone hysterectomy with removal of the cervix for benign indications [4]. Both the ACS and American College of Obstetricians and Gynecologists guidelines also state that women with a history of cervical intraepithelial neoplasia (CIN) 2 or worse should be screened annually until they have 3 negative vaginal Pap tests, after which screening can be stopped; however, women treated for cervical or vaginal cancer and women with a history of diethylstilbestrol (DES) exposure should continue screening for as long as

they do not have a life-limiting chronic condition [4, 7]. Studies have shown that the prevalence of significant abnormalities after total hysterectomy for benign disease is very low and that such screening is not cost-effective [5, 6]. However, with the growing trend to perform supracervical hysterectomies, accurate history is critical to decide whether a patient should still be screened.

Women who have received radiation and/or chemotherapy for gynecological cancer frequently have low-cellularity specimens with therapy-related changes. The main utility of cytology specimens in this setting is to detect recurrent malignancy. Expert opinion would suggest that such specimens should have many well-preserved cells or several cell groups, but there are no data to suggest a minimum numeric threshold at this time. Furthermore, studies have shown that cytological screening after treatment of endometrial cancer infrequently detects asymptomatic vaginal recurrences and may not be an effective surveillance tool [10]. Laboratories should exercise judgment in evaluating posttherapy cytology specimens based on the clinical setting and not rely solely on a specific numeric threshold.

The subset of women with unsatisfactory specimens due to inadequate squamous cellularity may include some older women with atrophic samples. If these women do not require screening because of negative history and risk factors, repeat screening may not be necessary. The ACS Guidelines state that women 70 years and older who have had 3 or more consecutive satisfactory negative Pap tests and no abnormal Pap tests in the last 10 years may choose to stop cervical cancer screening [7]. The positive predictive value of cervical cytology in previously screened postmenopausal women has been reported as low [11].

Many of the longitudinal studies of women with unsatisfactory Pap tests that showed a higher risk of epithelial abnormalities were performed before the Bethesda 2001 conference or used different cellularity criteria [8, 9]. The unsatisfactory Pap tests in these studies included many CSs with obscuring blood and inflammation. There are very few studies on the significance of insufficient squamous cellularity. One study performed using Bethesda 2001 criteria found that women with inadequate squamous cellularity on Pap tests do not have a significantly higher risk of abnormalities [12]; however, this study consisted mainly of CSs. Another study concluded that it was not possible to define a minimum acceptable squamous cellularity that would give an acceptable probability of detection of all LBPs containing abnormal cells [13]. This latter study suggested that a minimum cellularity threshold be set pragmatically by the screening program to provide a feasible percentage of repeat tests. The Bethesda 2001 squamous cellularity criteria provide an acceptable threshold of unsatisfactory results for most patient populations and laboratory settings, although additional studies and data would be useful.

Clinicians who receive a significant number of unsatisfactory reports from women after hysterectomy or after therapy are encouraged to discuss the lack of defined numeric criteria with the cytology laboratory director.

Issue 2: EC/TZ Component, Quality Indicators, and HPV Testing

The importance of the EC/TZ component in defining adequacy is controversial: research studies on the significance are conflicting [1]. While abnormal cells are more commonly found in specimens with an EC/TZ component [14], longitudinal studies have not shown that women with Pap smears lacking an EC/TZ component are at increased risk for developing high-grade squamous lesions and cancer [15]. Women with Pap smears lacking an EC/TZ component may represent a lower risk group as a whole because this group is skewed toward older ages [14, 16]. This may explain the apparent inconsistency between longitudinal studies, which suggest no increase in risk, and cross-sectional studies, which report more abnormalities in samples that contain an EC/TZ component. The proportion of endocervical adenocarcinoma among all cervical carcinoma cases is increasing, however [17]. Thus, the absence of an EC/TZ component may have greater significance in defining a Pap test's adequacy in detection of adenocarcinoma. Therefore, the committee opinion is that some women with Pap tests lacking an EC/TZ component may benefit from a shorter screening interval (CIII).

Women with a positive HPV test and a concurrent negative Pap test are at increased risk for future abnormalities [18]. In women with a positive HPV test, but lacking an EC/TZ component, there is the additional possibility of an increased risk of a false-negative cytology result in the presence of an endocervical adenocarcinoma or a squamous lesion high in the canal.

When the HPV test is negative, there may be more uncertainty regarding optimal management. Although the negative predictive value for women with a negative HPV test is high, most studies have not evaluated adequacy of HPV samples. Currently, there are little

published data from which to develop a morphological definition of an adequate sample for HPV testing. Inadequate samples may be due to lack of any cellular material (the specimen was inadvertently not transferred to the vial) or to samples showing low cellularity. There are currently very few studies examining the HPV status of women with negative cytology with, versus without, an EC/TZ component [19]. There are some data suggesting that hypocellular specimens in patients with high-grade lesions may have false-negative HPV results [20, 21]. Whether this may possibly reflect lack of sampling of the transformation zone is unknown. Conversely, HPV DNA may be detected in hypocellular specimens. Additional considerations include specific HPV types not included in the Hybrid Capture 2 (HC2) assay, DNA quantities below the threshold of the test, and other mechanisms. Both HPV prevalence and the reporting of EC/TZ component are inversely correlated with patient age, so that older women are more likely to be negative for both EC/TZ component and HPV [16]. Nevertheless, data from individual laboratories and a single published abstract appear to suggest that EC/TZ status and HPV DNA positivity appear to be independent variables [19]. Given the current uncertainty regarding the relationship between specimen adequacy parameters and HPV status, it is prudent to repeat the Pap test in 12 months in women older than 30 years with negative HPV and cytology lacking an EC/TZ component, rather than extending the interval to 3 years as recommended for women who are negative on both test results [4, 7].

Areas for Additional Research

The following topics represent areas in which significant clinical questions arise related to each of the issues above, but where there is little published evidence on which to base guidelines or recommendations.

Borderline Cellularity and Ensuring Adequate Sampling for HPV Testing. The current guidelines defining unsatisfactory specimens on the basis of low cellularity have been in use for several years. Whether lesser degrees of hypocellularity (5,000–20,000 squamous cells) are of clinical significance remains uncertain. In addition, the rising use of HPV testing—whether used as a follow-up test, coscreening test, or as a single primary screening test—has raised new issues regarding the degree of cellularity, specimen adequacy, and reliability of results for both Pap and HPV testing. These issues represent fertile ground for future investigation.

There are many physiological, anatomic, and clinical situations in which Pap test cellularity may be low or adequacy is considered compromised. These include mature and postmenopausal women, women who have undergone treatment for cervical disease, menstruating women, and others. In these clinical scenarios, how can the laboratory and the clinician ensure that there is adequate sampling for the HC2 HPV test or other HPV testing method if the test does not include a verification of specimen adequacy? Is a negative HPV result truly negative, or is there significant potential for false-negative results if there are few cells, questionable sampling of the EC/TZ, or other factors that may interfere with the test? When HPV testing without any internal adequacy validation step is performed in the absence of any morphological evaluation, as is the case in some follow-up situations, can one safely assume that the result is from an adequate sample? A few studies have begun to address these questions, but there is not yet a body of knowledge for definitive recommendations or practice guidelines. Nonetheless, cellularity, and endocervical cellularity, in particular, does appear to be a factor that influences reliability of HPV test results. Higher detection of oncogenic HPV viral load and increased detection of abnormalities have been demonstrated in cervical samples with larger numbers of endocervical cells [22]. Reports show that false-negative HPV tests occur more often in samples that contain few abnormal cells [20, 21]. Anatomic factors, such as a large cervical os, have also been noted to be associated with false-negative HPV tests, suggesting that this anatomic variation may adversely influence HPV sample adequacy [23]. The frequency of positive HPV tests by HC2 does not appear to vary with the date of last menstrual period, although there is a slightly higher frequency of HPV positivity in specimens collected at midcycle [24]. Although multiple studies have documented a very high negative predictive value of HC2 for high-grade lesions in various patient populations, these studies have not generally addressed sample adequacy. To provide optimal management guidelines for individual patients, studies addressing specimen adequacy for HPV testing are needed. For this to occur, future HPV testing methods will need to include an internal control.

Management of Women With Negative Cytology and HPV Results Which Lack an EC/TZ Component. Additional follow-up studies are needed to more rigorously determine if the presence or absence of an EC/TZ warrants different follow-up strategies in women

who have both negative Pap and HPV results. The most appropriate protocol may be age-dependent, so specific consideration of the following scenarios would potentially be beneficial as future areas of investigation:

- Women 30 years or older with no EC/TZ component and negative cytology and HPV cotest
- Can women lacking an EC/TZ component, but with multiple negative cytology results and negative HPV results, be returned to routine screening?
- In women younger than 30 years with no EC/TZ component present in repeated Pap tests, is there a role for HPV testing?
- In pregnant women with no EC/TZ component and negative cytology, would HPV testing of any value?

Sampling Adequacy for the Detection of Adenocarcinoma. Pap testing was originally designed for detection of cervical squamous cell carcinoma and its precursors; however, there is now an increased interest and expectation for the early detection and treatment of adenocarcinoma in situ (AIS) in the prevention of cervical adenocarcinoma. Until recently, the Pap test had not been demonstrated to have a major impact on the incidence of invasive adenocarcinoma [17, 25–31]. This had been attributed to suboptimal sampling of endocervical lesions and difficulties in recognition of the cytological features of the precursor lesion, AIS [32]. Several studies have reported either a rising incidence of adenocarcinoma in absolute numbers or a rising incidence relative to that of squamous carcinoma as the incidence of squamous carcinoma continues to decline [17, 28, 33–37]. Recent evidence suggests that modification in the sampling and technique of the Pap test may decrease the incidence of invasive adenocarcinoma [38–40]. For example, one large opportunistic screening program has reported a decline in the incidence of adenocarcinoma over a 10-year period (following a lengthy period of rising incidence) after the adoption of a dual sampling technique, appropriate terminology, and quality assurance efforts [38]. The use of LBP may be another factor in improving the detection of AIS and of adenocarcinoma. Although classic morphological features of AIS may not be as pronounced on LBP, a few studies suggest that LBP may have higher sensitivity in the detection of adenocarcinoma in comparison to conventional cytology [41, 42]. Current adequacy criteria have been largely defined with reference to the detection of squamous lesions only. It may be appropriate in the future to consider whether these current criteria are also optimal for the detection of glandular neoplasia or whether modification of these criteria is desirable. Finally, data are needed on the utility of HPV screening in the detection of AIS and adenocarcinoma as well.

Adequacy of Vaginal Pap Tests. Approximately 15% of US Pap tests are vaginal samples [43]. As discussed, the Bethesda System allows laboratories to exercise judgement in reporting cellularity of vaginal Pap tests and of those obtained after cancer therapy [2]. No peer-reviewed published studies are currently available to precisely establish what constitutes an adequate vaginal Pap sample. Most studies have concluded that vaginal screening after hysterectomy for benign disease is not cost-effective [5], but reliability of patient history and the risk of development of new vaginal lesions in sexually active posthysterectomy women have received little study. Most literature recommends continued cytological surveillance in women who have had hysterectomy for CIN or cervical cancer [4]. A recent review concluded that the value of vaginal cytology is largely unproven but that "inconsistency of study design and limited methodological quality mean that the value of vaginal vault smears could not be established" [44]. There is also a need to assess the possible utility of cytology and high-risk HPV DNA cotesting in this population because HPV types prevalent in vaginal intraepithelial neoplasia parallel those prevalent in CIN [45]. Additional research is needed on the value of vaginal cytology in patients with a benign history, what constitutes an adequate posthysterectomy vaginal specimen, an adequate posttherapy cervical/vaginal specimen, and the role of HPV cotesting in vaginal and posttherapy specimens.

Sampling Techniques to Improve Adequacy and Decrease False-Negative Rate

False-negative cytology is the term applied when the Pap test result does not accurately reflect the state of disease present in the cervix. False-negative cytology comprises "true" false-negatives (70%–80%) and laboratory errors (20%–30%) [46]. True false-negative Pap smears are free of abnormal cells, even on review of the slide, yet there is a histological evidence of cervical disease. These are classified as "sampling errors." Sampling errors may have multiple possible etiologies: inadequate patient preparation, sampling technique errors, and intermittent or inadequate shedding of abnormal cells. There is a general agreement that optimizing both patient preparation and sampling techniques is one strategy that will

reduce but not completely eliminate false-negative cytology results.

Optimal sample collection and adequate fixation are the most important factors in improving the reliability of cytology and reducing sampling errors. Steps taken by the clinician, from patient education to improved sampling technique, may help ensure that the sample collected maximizes the potential of the Pap test. Cervical cytology specimens are smeared directly onto a glass slide in the office (CS) or transferred to a liquid-based Pap medium (LBP). Most issues related to optimizing the sample are shared by each technique, but there are some differences that will be discussed.

General Recommendations for Optimal Sample Collection

Patient Preparation. When possible, patient education regarding preparation for obtaining an optimal Pap test should begin before the patient's office visit [47, 48]. This could be in the form of advice given over the phone at the time the appointment is scheduled, materials sent to the patient before the scheduled examination, or an information "alert" that "pops up" when online appointments are scheduled by the patient. The woman should be counseled to refrain from intercourse, douching, using tampons, or using intravaginal medication, for at least 48 hours before the examination to decrease the possibility that the number of exfoliated cells will be diminished or obscured by lubricants or spermicides [7, 47]. In addition, the patient should avoid scheduling her appointment during heavy menstrual bleeding, but should not defer for abnormal bleeding [7].

Labeling and Documentation. The first criterion for accurate assessment and reporting is the correct identification of the patient's specimen. When the slide or liquid-based vial is not labeled appropriately, the Pap will not be processed and will be reported as "rejected due to lack of patient identification" [2]. In this circumstance, the Pap test specimen must be repeated, as it cannot be labeled retrospectively. For a CS, the frosted end of the slide must be clearly labeled in pencil or indelible marker before the specimen is collected. For LBP, the vial should be clearly labeled before obtaining the specimen. In either case, 2 unique patient identifiers (name and either date of birth or patient identification number) should be included on the label for proper specimen identification. Before taking the sample for a CS, the collection device(s) and either spray or liquid-pour fixative or an open bottle of 95% ethanol should be ready for immediate fixation of the slide; rapid fixation reduces the possibility of drying artifact which may reduce the quality of the Pap test [47]. Air-drying artifact typically does not occur with liquid-based cytology.

Pertinent Clinical History. In addition to patient age and date of last menstrual period, other relevant clinical history and observations should be provided (e.g., a history of prior abnormal Pap smears, suspicious findings, prior cervical treatment, or other factors that increase the risk for cervical neoplasia) [48]. These "high-risk" Pap smears are typically rescreened by another cytologist as a quality control measure. Additional history that may prove helpful includes current pregnancy, use of hormonal contraceptives or estrogen replacement therapy, or presence of an intrauterine device [47].

Choosing the Optimal Collection Device. There are numerous sampling devices available to collect Pap tests. In general, these can be referred to as spatulas, brushes, swabs, and brooms. A recent meta-analysis of numerous studies on collection devices and specimen adequacy supported the use of the extended-tip spatula and devices that effectively collect endocervical cells [14]. However, the cotton swab traps cells and results in the greatest number of Pap smears with cellular distortion and the fewest endocervical cells [49, 50]. With liquid sampling, one should use the sampling device(s) approved for that particular LBP.

Because the goal of the Pap is to sample the entire transformation zone, the choice of collection device should be tailored to the clinical appearance of the cervix. For example, a large broom device may provide a more representative sample when the patient has a large ectopy with a peripheral or large transformation zone. In contrast, in postmenopausal women and in women with prior cervical excisional or ablative treatment, the squamocolumnar junction is usually located within the endocervical canal and the cervical os may be narrow; sampling with an endocervical brush plus spatula may be more appropriate. Women after cervical laser sampled with an endocervical brush plus an Ayre spatula were shown to have better quality Pap results than those sampled with the broom alone, in regard to both adequacy and the presence of endocervical cells [51].

Optimal Technique. The cytology specimen should be taken before the bimanual examination [47]. An

appropriately sized speculum should be inserted with either water or sparing use of a lubricant gel applied to facilitate insertion. Water-soluble gel lubricant sparingly applied to the posterior blade of the speculum to ease insertion does not result in an increased rate of unsatisfactory CS [52, 53]. However, lubricants can cause an unsatisfactory LBP, especially the ThinPrep Pap [54].

It is critical to fully expose the cervix by manipulation of the speculum or the cervix to obtain the most direct frontal view of the portio and cervical canal before collecting the Pap sample; otherwise, taking the endocervical sample may be compromised. Excessive mucus, discharge, or menstrual blood can be gently removed or blotted from the cervix before taking the sample. When there is an evidence of significant infection or inflammation, consideration should be given to treatment before taking the Pap, unless there are findings suspicious for cervical neoplasia or the patient cannot be relied on to return [47].

Previously, it has been recommended that the Pap sample should be collected before the collection of samples for sexually transmitted infection [47]. However, recent studies have documented that when more than 1 cervical specimen is collected for sexually transmitted infections and for cervical cytology, the proportion of inadequate CS and LBP are independent of specimen order [55–57].

When 2 sampling devices are used to collect the Pap, the exocervical sample is obtained first, followed by the endocervical sample. This minimizes contamination by bleeding that may accompany the use of the endocervical brush. The spatula should be rotated at least 360 degrees around the ectocervix as per the manufacturer's instructions; the broom should be rotated 5 times. To fully sample the entire transformation zone in some women, passing the sampling device across areas of the transformation zone that fall outside the area covered by the central 360-degree rotation may also be required as well [47].

Lesion size and location have been reported to be important determinants in sampling error [58, 59]. Lesions on a women's right ectocervix have been shown to be associated with more frequent false-negative results than lesions on left [59]. This may be secondary to the tendency of the sampling device to lift off the cervix during the part of the rotation in which the rotating hand necessarily inverts. That this more often occurs on the right side of the cervix is due to the majority of the population being right-handed. For left-handed clinicians, lesions on the left-hand side of the cervix would be at greatest risk of poor sampling. Hence, care must be taken by right-handed individuals to pass a second sweep of the spatula over the right portio of the cervix and by those left-handed to do the same over the left portio.

Another study demonstrated that the location of the squamocolumnar junction, the size of the transformation zone area, the size of the acetowhite area, and the ratio of the acetowhite area to the area of the transformation zone influenced the accuracy of cytology [58]. In particular, women with large transformation zone areas (>30.03 mm^2) and/or small acetowhite lesions (<7.01 mm^2) are more likely to have an inaccurate cytology report than women with small transformation zones and women with larger acetowhite areas. These data confirm the importance of clinically evaluating the architecture of the cervix before taking the Pap specimen to optimally tailor the sample collection for each individual patient. Some individuals have used additional sampling devices or prepared split CSs and LBPs in women who have a history of unsatisfactory results due to low squamous cellularity, especially in the setting of atrophy or postpartum state. Such approaches may help minimize repeat unsatisfactory reports.

The endocervical brush, if used, should not be inserted into the endocervix beyond the full length of the brush and should be turned in the canal no more than 180 degrees to minimize bleeding [47]. If the cervix is shortened because of prior surgery, clinicians should be aware that sampling of the endometrium can lead to cytological interpretation challenges and should refrain from deep brush insertion. The use of the brush during pregnancy has been shown to be safe and effective [60, 61].

Each specimen collected for an LBP should be immediately immersed in the liquid medium as soon as it is obtained. In contrast, when 2 sampling devices are used for a CS, the spatula sample should be held until the endocervical specimen is obtained and each then is sequentially smeared on the slide, followed by immediate fixation [47]. Prevention of drying artifact is critical and is best achieved by rapid fixation in 95% ethanol solution or by quickly spraying or covering the slide with a poured-on liquid fixative. A spray fixative is best applied by holding the spray nozzle approximately 9 to 12 inches from the slide. A single-slide technique for CS, rather than 2 slides, is preferable; it does not result in a diminished detection rate, and it uses less laboratory materials and screening time [62, 63].

Rarely, a separate vaginal Pap specimen is needed for the follow-up of women with a previous history of vaginal lesions or for DES-exposed women. In these circumstances an Ayre spatula or cervical brush can be used to obtain vaginal samples for either CS or LBPs. For DES-exposed women, a sample from each lateral vaginal wall should be collected [47]. The speculum is then rotated, and the procedure repeated on the anterior and posterior vaginal walls. Specimens should be placed on separate slides or containers if the specific anatomic location of the sampling is desired. Gynecological cytology is no longer recommended for hormonal evaluation.

CONCLUSION

Specimen adequacy management guidelines should help promote uniform and optimal follow-up of patients receiving Pap tests. However, data to determine recommendations are lacking or conflicting in many areas, and research studies are needed to define future management options. Clinicians performing Pap tests are encouraged to adopt optimal sampling techniques tailored to the individual patient to minimize false-negative results.

REFERENCES

1. Davey DD, Austin RM, Birdsong G, Buck HW, Cox JT, Darragh TM, et al. ASCCP patient management guidelines: Pap test specimen adequacy and quality indicators. *J Low Genit Tract Dis* 2002;6:195–9.

2. Birdsong GG, Davey DD, Darragh TM, Elgert PA, Henry M. Specimen adequacy. In: Solomon D, Nayar R, eds. *The Bethesda System for Reporting Cervical Cytology.* Springer-Verlag: New York; 2004:1–20.

3. Wright TC, Cox JT, Massad LS, Twiggs LB, Wilkinson EJ. 2001 ASCCP-Sponsored Consensus Conference. 2001 Consensus guidelines for the management of women with cervical cytological abnormalities. *JAMA* 2002;287:2120–9.

4. ACOG Practice Bulletin No. 45. Clinical management guidelines for obstetrician-gynecologists: cervical cytology screening. *Obstet Gynecol* 2003;102:417–27.

5. Fetters MD, Lieberman RW, Abrahamse PH, Sanghvi RV, Sonnad SS. Cost-effectiveness of Pap smear screening for vaginal cancer after total hysterectomy for benign disease. *J Low Genit Tract Dis* 2003;7:194–202.

6. Pearce KF, Haefner HK, Sarwar SF, Nolan TE. Cytopathological findings on vaginal Papanicolaou smears after hysterectomy for benign gynecologic disease. *N Engl J Med* 1996;335:1559–62.

7. Saslow D, Runowicz CD, Solomon D, Moscicki AB, Smith RA, Eyre HJ, et al. American Cancer Society guideline for the early detection of cervical neoplasia and cancer. *CA Cancer J Clin* 2002;52:342–62.

8. Hock YL, Ramaiah S, Wall ES, Harris AM, Marston L, Marshall J, et al. Outcome of women with inadequate cervical smears followed up for five years. *J Clin Pathol* 2003;56: 592–5.

9. Ransdell JS, Davey DD, Zaleski S. Clinicopathologic correlation of the unsatisfactory Papanicolaou smear. *Cancer (Cancer Cytopathol)* 1997;81:139–43.

10. Cooper AL, Dornfeld-Finke JM, Banks HW, Davey DD, Modesitt SC. Is cytologic screening an effective surveillance method for detection of vaginal recurrence of uterine cancer? *Obstet Gynecol* 2006;107:71–6.

11. Sawaya GF, Grady D, Kerlikowske K, La Valleur J, Barnabei VM, Bass K, et al. The positive predictive value of cervical smears in previously screened postmenopausal women: the Heart and Estrogen/Progestin Replacement Study (HERS). *Ann Intern Med* 2000;133:942–50.

12. Adams AL, Gidley J, Roberson J, Wang W, Eltoum I, Chhieng DC. Clinical significance of unsatisfactory conventional smears owing to inadequate squamous cellularity defined by the Bethesda 2001 criterion. *Am J Clin Pathol* 2005;123:738–43.

13. McQueen F, Duvall E. Using a quality control approach to define an adequately cellular liquid-based cervical cytology specimen. *Cytopathology* 2006;17:168–74.

14. Martin-Hirsch P, Lilford R, Jarvis G, Kitchener H. Efficacy of cervical-smear collection devices: a systematic review and meta-analysis. Lancet 1999;354:1763–70.

15. Mitchell HS. Longitudinal analysis of histologic high-grade disease after negative cervical cytology according to endocervical status. *Cancer (Cancer Cytopathol)* 2001;93: 237–40.

16. Baer A, Kiviat NB, Kulasingam S, Mao C, Kuypers J, Koutsky LA. Liquid-based Papanicolaou smears without a transformation zone component: should clinicians worry? *Obstet Gynecol* 2002;99:1053–9.

17. Smith HO, Tiffany MF, Qualls CR, Key CF. The rising incidence of adenocarcinoma relative to squamous cell carcinoma of the uterine cervix in the United States: a 24-year population-based study. *Gynecol Oncol* 2000;78: 97–105.

18. Castle PE, Wacholder S, Sherman ME, Lorincz AT, Glass AG, Scott DR, et al. Absolute risk of a subsequent abnormal Pap among oncogenic human papillomavirus DNA-positive, cytologically negative women. *Cancer* 2002; 95:2145–51.

19. Navina S, Savid K, Miller L, McKeon D, Wang X, Gulbahce E, et al. The effect of the presence of an adequate EC/TZ component on the detection of the high risk HPV (hr-HPV) DNA. A study of 7,990 cases classified by the 2001 Bethesda System. *Cancer (Cancer Cytopathol)* 2006;108:397.

20. Jastania R, Geddie WR, Chapman W, Boerner S. Characteristics of apparently false negative digene hybrid

capture 2 high-risk HPV DNA testing. *Am J Clin Pathol* 2006;125:223–8.

21. Eltoum IA, Chhieng DC, Crowe DR, Roberson J, Jin G, Broker TR. Significance and possible causes of false-negative results of reflex human papillomavirus testing. *Cancer (Cancer Cytopathol)* 2007;111:154–9.

22. Depuydt CE, Benov IH, Bailleul EJ, Vandepitte J, Vereecken AJ, Bogers JJ. Improved endocervical sampling and HPV viral load detection by Cervex-Brush Combi. *Cytopathology* 2006;17:374–81.

23. Zuna RE, Wang SS, Rosenthal DL, Jeronimo J, Schiffman M, Solomon D, et al. Determinants of human papillomavirus-negative low-grad squamous intraepithelial lesions in the atypical squamous cells of undetermined significance/low-grade squamous intraepithelial lesions triage study (ALTS). *Cancer (Cancer Cytopathol)* 2005;105:253–62.

24. Sherman ME, Carreon JD, Schiffman M. Performance of cytology and human papillomavirus testing in relation to the menstrual cycle. *Br J Cancer* 2006;94:1160–6.

25. Anderson S, Larson B, Hjerpe A, Silfversward C, Sallstrom J, Wilander E, et al. Adenocarcinoma of the uterine cervix: the presence of human papillomavirus and the method detection. *Acta Obstet Gynecol Scand* 2003;82:960–5.

26. Bergstrom R, Sparen P, Adami H-O. Trends in cancer of the cervix uteri in Sweden following cytological screening. *Br J Cancer* 1999;81:159–66.

27. Bulk S, Visser O, Rozendaal L, Veerheijen RH, Meijer CJ. Incidence and survival rate of women with cervical cancer in the Greater Amsterdam area. *Br J Cancer* 2003;89:834–9.

28. Liu S, Semenciw R, Probert A, Mao Y. Cervical cancer in Canada: changing patterns in incidence and mortality. *Int J Gynecol Cancer* 2001;11:24–31.

29. Mitchell H, Medley G, Gordon J, Giles G. Cervical cytology reported as negative and risks of adenocarcinoma of the cervix: no strong evidence of benefit. *Br J Cancer* 1995;71:894–7.

30. Nieminen P, Kallio M, Anttila A, Hakama M. Organized vs. spontaneous Pap-smear screening for cervical cancer: a case-control study. *Int J Cancer* 1999;83:55–8.

31. Wang SS, Sherman ME, Hildesheim A, Lacey JV, Devesa S. Cervical adenocarcinoma and squamous cell carcinoma incidence trends among white women and black women in the United States for 1976–2000. *Cancer* 2004;100: 1035–44.

32. Renshaw AA, Mody DR, Lozano RL, Volk EE, Walsh MK, Davey DD, et al. Detection of adenocarcinoma in situ of the cervix in Papanicolaou tests: comparison of diagnostic accuracy with other high-grade lesions. *Arch Pathol Lab Med* 2004;128:153–7.

33. Alfsen GC, Thoresen SO, Kristensen GB, Skovlund E, Abeler VM. Histopathologic subtyping of cervical adenocarcinoma reveals increasing incidence rates of endometrioid tumors in all age groups. *Cancer* 2000;89:1291–9.

34. Armstrong B, Holam D. Increasing mortality from cancer of the cervix in young Australian women. *Med J Aust* 1981;1:460–2.

35. Sasieni P, Adams J. Changing rates of adenocarcinoma and adenosquamous carcinoma of the cervix in England. *Lancet* 2001;357:1490–3.

36. Vizcaino AP, Moreno V, Bosch FX, Munoz N, Barros-Dios XM, Parkin DM. International trends in the incidence of cervical cancer. I. Adenocarcinoma and adenosquamous cell carcinomas. *Int J Cancer* 1998;75:536–45.

37. Zheng T, Holford TR, Ma Z, Chen Y, Liu W, Ward BA, et al. The continuing increase in adenocarcinoma of the uterine cervix: a birth cohort phenomenon. *Int J Epidemiol* 1996;25:252–8.

38. Howlett R, Marrett L, Innes M, Rosen B, McLachlin CM. Decreasing incidence of cervical adenocarcinoma in Ontario: is this related to improved endocervical Pap test sampling? *Int J Cancer* 2006;120:362–7.

39. Mitchell H, Hocking J, Saville M. Improvement in protection against adenocarcinoma of the cervix resulting from participation in cervical screening. *Cancer (Cancer Cytopathol)* 2003;99:336–41.

40. Romero AA, Key CR, Smith HO. Changing trends in adenocarcinoma and squamous cell carcinoma by age, race and stage: a 25-year population-based study. *Gynecol Oncol* 2001;80:295–6.

41. Hecht JL, Sheets EE, Lee KR. Atypical glandular cells of undetermined significance in conventional cervical/vaginal smears and thin-layer preparations. *Cancer (Cancer Cytopathol)* 2002;96:1–4.

42. Schorge JO, Saboorian MH, Hynan L, Ashfaq R. ThinPrep detection of cervical and endometrial adenocarcinoma. *Cancer (Cancer Cytopathol)* 2002;96:338–43.

43. Solomon D, Breen N, McNeel T. Cervical cancer screening rates in the United States and the potential impact of implementation of screening guidelines. *CA Cancer J Clin* 2007;57:105–11.

44. Stokes-Lampard H, Wilson S, Waddell C, Ryan A, Holder R, Kehoe S. Vaginal vault smears after hysterectomy for reasons other than malignancy: a systematic review of the literature. *BJOG* 2006;113:1354–65.

45. Srodon M, Stoler MH, Baber GB, Kurman RJ. The distribution of low and high-risk HPV types in vulvar and vaginal intraepithelial neoplasia (VIN and VaIN). *Am J Surg Pathol* 2006;30:1513–8.

46. Nielsen ML, Davey DD, Kline TS. Specimen adequacy evaluation in gynecologic cytopathology: current laboratory practice in the College of American Pathologists Interlaboratory Comparison Program and tentative guidelines for future practice. *Diagn Cytopathol* 1993;9: 394–403.

47. Cox JT. ASCCP practice guideline: management issues related to quality of the smear. *J Lower Gen Tract Dis* 1997;2: 100–6.

48. Arbyn M, Herbert A, Schenck U, Nieminen P, Jordan J, Mcgoogan E, et al. European guidelines for quality assurance in cervical cancer screening: recommendations for collecting samples for conventional and liquid-based cytology. *Cytopathology* 2007;18:133–9.

49. Germain M, Heaton R, Erickson D, Henry M, Nash J, O'Connor D. A comparison of the three most common Papanicolaou smear detection techniques. *Obstet Gynecol* 1994;84:168–73.

50. Lai-Goldman M, Nieberg RK, Mulcahy D, Wiesmeier E. The cytobrush for evaluating routine cervicovaginal-endocervical smears. *J Reprod Med* 1990;35:959–63.

51. Szarewski A, Cuzick J, Singer A. Cervical smears following laser treatment. Comparison of Cervex brush versus cytobrush-Ayre spatula sampling. *Acta Cytol* 1991;35:76–8.

52. Amies AM, Miller L, Lee SK, Koutsky L.The effect of vaginal speculum lubrication on the rate of unsatisfactory cervical cytology diagnosis. *Obstet Gynecol* 2002;100: 889–92.

53. Griffith WF, Stuart GS, Gluck KL, Heartwell SF. Vaginal speculum lubrication and its effects on cervical cytology and microbiology. *Contraception* 2005;72:60–4.

54. Prey MU. Q and A column. *CAP Today.* May 2007: 90–3.

55. Ghanem KG, Koumans EH, Johnson RE, Sawyer MK, Papp JR, Unger ER, et al. Effect of specimen order on *Chlamydia trachomatis* and *Neisseria gonorrhoeae* test performance and adequacy of Papanicolaou smear. *J Pediatr Adolesc Gynecol* 2006;19:23–30.

56. Hildesheim A, Bratti CM, Edwards RP, Schiffman M, Rodriguez AC, Herrero R, et al. Collection of cervical secretions does not adversely affect Pap smears taken immediately afterward. *Clin Diagn Lab Immunol* 1998;5:491–3.

57. Marchand L, Mundt M, Klein G, Agarwal SC. Optimal collection technique and devices for a quality Pap smear. *WMJ* 2005;104:51–5.

58. Al-Awadhi RM, Mansell E, Chong S, Chow C, Singer A, Coleman DV. Video monitoring of smear-taking at colposcopy: relationship to cytology. *BJOG* 2004;111: 967–73.

59. Jeronimo J, Castle PE, Herrero R, Sherman ME, Bratti MC, Hildesheim A, et al. Right-sided ectocervical lesions may be associated with false-negative cytology among women with histologic cervical intraepithelial neoplasia 2 or 3. *J Low Genit Tract Dis* 2003;7:175–83.

60. Orr JW, Barrett JM, Orr PF, Holloway RW, Holiman JL. The efficacy and safety of the cytobrush during pregnancy. *Gynecol Oncol* 1992;44:260–2.

61. Paraiso MF, Brady K, Helmchen R, Roat TW. Evaluation of the endocervical cytobrush and Cervex-brush in pregnant women. *Obstet Gynecol* 1994;84:539–43.

62. Pinto MM, Waradzin M. One-slide Papanicolaou smears prepared using combined cytobrush-spatula sampling. *Acta Cytol* 1989;33:705.

63. Wilbanks GD, Ikomi E, Prado RB, Richart RM. An evaluation of a one-slide cervical cytology method for the detection of cervical intraepithelial neoplasia. *Acta Cytol* 1968;12;157–8.